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# *The Journal of Laboratory and Clinical Medicine*

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## *CLINICAL AND EXPERIMENTAL*

### THE THERAPEUTIC USE OF BACTERIOPHAGE IN SUPPURATIVE CONDITIONS\*

REPORT OF FIFTY CASES

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INDIANAPOLIS IND

AS A LABORATORY phenomenon the bacteriophage is now an accepted fact though its exact nature is far from being definitely settled. As a therapeutic measure however, there is much less unanimity in spite of the fact that d'Helle has made large claims for it almost from the first. The following series of cases is reported in the hope that so important a matter may be brought into more general application if proved successful. We have used the bacteriophage in a somewhat different manner than is commonly recommended. The results have been gratifying in most of the fifty cases which have been treated.

The manner of preparing the bacteriophage filtrate is important and we are of the opinion that most of the failures described by various writers and experimenters have been due to the inactivity of the strain of bacteriophage used for the particular strain of bacteria in the lesions under treatment. In each of the cases here described there was active though not necessarily complete lysis of the autogenous culture *in vitro*. (An exception to this rule is made in a few of the cases with very acute infections as boils which did not give time for an autogenous technique. In these cases material prepared for a similar case was used first, and then if time permitted before the cure was complete the autogenous preparation was used later.)

Our strains of bacteriophage have been obtained from various sources but mostly they have originally been isolated from mixed sewage and then "trained" to activity by being grown with the particular organism against which lytic action was desired. When a new case was taken, various strains of bacteriophage were tried with the autogenous culture, and the most active

one chosen. In more recent work we have mixed a number of active strains and have added this mixture to the bacterial culture. Considerable activity has then usually been obtained at the first attempt. The culture medium that we have used has been either hormone broth or veal infusion broth with a hydrogen-ion concentration of 7.6. We have used Seitz filters instead of the Berkefeld candles, and have found them more satisfactory than the latter.

Our efforts have been confined to infections due to *Staphylococcus aureus*, *Staphylococcus albus*, *Escherichia coli* (*Bacillus coli*), and *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*). Best results have been observed in those cases for which we have obtained the best lysis, and as a rule the poor results have been seen when the bacteriophage showed low ability *in vitro*. Early in the series we gave part of the material by subcutaneous injection but for the most part we have used the bacteriophage filtrate as a wet dressing applied directly to the lesion, or as an instillation into a sinus, an abscess cavity, or into the urinary bladder. In two cases the material was injected into an unopened abscess with unusually good results. Many of our cases have been dressed two or three times a day for weeks with the filtrate poured directly into the wound or impregnating the gauze in contact with the tissues. In this respect we have differed rather widely from the technic commonly used.

There are four possibilities to be considered in explaining our results:

- 1 That the good results are due to the destruction of the offending organisms by the bacteriophage according to the common interpretation of the Twort-d'Herelle action.

- 2 That the result is due to the antiviral action as described by Besredka. In every bacteriophage filtrate as commonly prepared there are, of course, the metabolic products of bacterial growth, which means that to that extent the filtrate is an antiviral preparation. We are conducting another series of experiments at the present time in which the bacteriophage is not being added to the cultures, and are getting results similar to those reported by Besredka and his followers. It may be that bacteriophage is also present in these preparations. This series will be reported later.

- 3 That the action is due to the presence of an unusually efficient and available form of the bacterial antigen which has been formed by the lysis of the causative organism by the bacteriophage.

- 4 Finally it is by no means impossible that the result is due to a combination of these various properties, or that indeed all are but different manifestations of the same thing. There is also the possibility that the broth itself exerts a beneficial effect.

The following cases represent all that we have treated with the exception of a few who died of their primary condition or left the hospital before there had been time for the effect of the therapy. It should be understood that early in our experiments we were given only cases that were considered to be quite hopeless, and that several of these patients died under circumstances which cast no reflections upon bacteriophage therapy. Some of the cases are of little value in this report, but are included in order that our poorest as

well as our best results may be evaluated. The cases are grouped according to the type of pathology encountered.

**Boils**—In all cases of boils treated the organism was shown to be *Staphylococcus aureus* and the bacteriophage strain used was active *in vitro*. The tubes were rarely cleared to the degree commonly observed when *Bacillus coli* was used, but active bacteriophage action was considered to be present if the culture showed the marked characteristics of a "rough" *staphylococcus* growth as compared with the control culture of the same organism without bacteriophage, i.e., when self agglutination more or less clearing and a yellowish coloring of the broth were observed.

The following results with minor variations were observed in nearly all of the cases treated. (1) The boils were aborted when the filtrate was applied early in their development. (2) If already well developed they went through their evolution more rapidly than is commonly the case. (3) The pus coming from them was usually liquid, and when evacuated left a clean smooth crater. (4) Pain was usually promptly relieved, and the lesions showed diminished soreness after a few hours at the most. (5) The lesions were promptly healed with little induration and there was rarely recurrence.

It is interesting to find Haudurov, Camus and Dalsace referring to "a very marked diminution of pain, a rapid disappearance of erythema, the early softening of the induration, the reabsorption of the edema, and a rapid liquification of the purulent material" (close translation from the French, *Presse med* Sept 22, 1926) as following closely the use of the bacteriophage in the treatment of boils. We had observed the same phenomena exactly many times before reading their article.

The prompt relief from pain following this treatment requires special attention. It is so striking and often so complete that one who has not observed it may be pardoned for being skeptical. There are, however, numerous references in the literature to observations even more striking than those we are reporting. Several of these references are to the use of antiviral but we are inclined to believe that the two phenomena are related, and we have observed the same effect after the use of both bacteriophage and antiviral filtrates.

We have repeatedly seen patients enter the laboratory guarding the seat of a boil or carbuncle in the most careful manner in order to avoid jars and bumps. After the application of a wet bacteriophage dressing for as short a time as ten or fifteen minutes, or more commonly after an hour or two, we have frequently been able to squeeze the lesion rather roughly without causing complaint. Many have remarked, entirely without suggestion from our selves, that the pain and throbbing have stopped in a short time and that muscular action which had before been unbearable had become easy.

#### CASE REPORTS BOILS

**CASE 7**—H. A. (City Dispensary), male, aged thirty eight had nine large boils on his left forearm. Bacteriophage prepared for another case of boils was used. The patient was given enough of the material to last for three days after which he was to return for the autogenous preparation. He did not return for three weeks having been "cured" by

the treatment. When he finally came back he had had a recurrence, and presented fifteen rather large boils and two carbuncles on the hands and forearms, except that there were none on the left forearm on the area previously treated. When the autogenous preparation previously made was applied there was immediate relief from pain and soreness, and a complete cure with no new lesions after eleven days. Urine examination showed large amounts of sugar, and there was clinical evidence of diabetes.

CASE 6—Whar, a student nurse, had been having boils for months. At the time that treatment was begun she had a number of large and painful boils. There was complete cure in one week. One boil was injected with the filtrate and promptly retrogressed. The injection was of course very painful and for this reason wet dressings were mostly used. The patient has had a few boils since, but they have been smaller, less painful and mostly in locations not treated locally.

CASE 14—Baby K (Riley Hospital, No 4312), male, aged five months. This child began at the age of three months to have boils. When first seen by us two months later, it was covered from head to foot with them, there being between 250 and 300 definite lesions of various sizes. Several of these were a centimeter or more in diameter, and a few were twice that size. They covered practically the entire body making the skin fiery red, and almost boardlike. The baby was irritable, thin, and whimpering constantly. It was unable to sleep and was running some temperature. Filtrate prepared for another case was first used while the autogenous preparation was coming on. The following day the child was better and more comfortable. After another day the improvement was marked, the child being able to sleep well, and to laugh and coo when awake. After eight days all but the large lesions were gone and the skin was soft. A few days later there was but one boil, and the child was gaining rapidly in weight and strength. The child was discharged completely cured and in excellent condition twenty-three days after our treatment began.

CASE 15—V W, a medical student, aged twenty-two, was subject to occasional boils. An unusually severe one came in the right nostril. He had been cautioned to avoid manipulation. It was necessary to take large doses of salicylates to keep down the pain, but even so he was pacing the floor unable to sleep, study, or attend classes. A pledget of absorbent cotton was saturated with the bacteriophage and inserted into the nostril, being replaced every half hour. Another similar pledget was placed over the tip of the nose. Absolutely all pain was gone after three and one-half hours. The next morning even the soreness was gone except for one little spot. During the second day the boil opened and about five drops of liquid pus was evacuated, and in forty-eight hours all signs of the boil had disappeared.

CASE 21—Dr O, a prominent surgeon had for six months been subject to large numbers of painful boils on the left forearm. He had tried "everything" and had gotten no relief. Bacteriophage suspended in a soft agar gel was given him for local application. He reported that pain was relieved, that the boils were less sore, and were smaller. After about two months of treatment he was free of them. Treatment was discontinued for about a month and at the end of this time there was recurrence (six boils).

CASE 23—C K, male, aged twenty-one, a patient of Dr H. This man had a pimple under his left arm. He applied a poultice to it, and then shaved the axilla to remove the matted hair and poultice material. A large carbuncle-like lesion developed in the axilla and surrounding region as a result of his nicking the skin with the razor. The patient was in serious condition, had high fever, and was delirious. The usual means of treatment were giving no improvement. Bacteriophage was applied December 22. There was immediate improvement, and the case was dismissed cured on January 2. The attending surgeon's words describing the case are as follows: "Induration receded. The axilla is in such condition as is usual only months after such an inflammatory process. Full credit is given to the bacteriophage."

CASE 30—F L (Industrial Clinic), male, aged twenty. This man was a worker in silica, and probably as a result had a great number of large and painful boils, about fifty, on each forearm. There were also several on the legs and a few on the abdomen. After three treatments the man was so improved that he did not come back, and was not seen for a week. At this time he was much improved being practically well except for two or three of the larger lesions which were healing. There were no new boils.

CASE 31—Mr B, medical student, had a large boil or small carbuncle on the back of his neck. There was prompt relief from pain upon application of the bacteriophage in the form of a wet dressing. It improved rapidly and was healed. About ten days later he came back with another unusually deep lesion in the same location. This time the bacteriophage was ineffectual, probably because the seat of the trouble was deep in a thick layer of fat and subcutaneous tissue. We proposed to inject the filtrate into it with a needle, but permission was not granted.

CASE 32—J S, male, aged seventeen, has had many large boils on his face in recent months, and also a marked pustular acne. His general physical condition was found to be bad and his urine showed a large amount of albumin, three-plus, on several occasions. He was sent to a genitourinary specialist who in turn sent him back to us thinking that the kidney condition was secondary to the infection of his face. With specific wet dressings and bacteriophage suspended in soft agar applied to the face for a period of six weeks the face is now nearly clear; there have been no more boils, and there is barely a trace of albumin found occasionally in the urine. His general health appears to have improved.\*

CASE 36—Dr K had a carbuncle on the back of his neck. Wet dressings of bacteriophage filtrate were applied. There was relief of pain after about ten minutes. This treatment was witnessed by four other physicians who were skeptical, but who will now vouch for the above statement. The core came out two days later leaving a clean crater and the boil healed promptly without further pain.

CASE 41—J K, male, aged fifty-five, had two boils in the axilla, one of them well developed and the other only started. There was immediate relief, and a complete cure in three days.

CASE 43—A D, female, aged eighteen, had a painful sty. There was prompt relief from pain; the pus was evacuated after a few hours, and a condition that gave promise of being severe was well in less than two days.

CASE 39—Mrs S, aged thirty-one, frequently has had superficial boil-like lesions on the face. They were painful and unsightly, leaving a red blotch which would persist for a long time. Bacteriophage wet dressings were followed by prompt healing, and when applied early seemed to abort the infection. She has had none for several weeks.

*Abscesses*—The treatment of abscesses has been much like that of boils except that the bacteriophage filtrate has been injected into the opened or unopened abscess, or used to saturate gauze dressings which were then packed into the cavity. Mixed infections were treated with mixed bacteriophage filtrates prepared separately at first, but later it was found that it was more convenient to grow the material in mixed culture and prepare the material by one manipulation. Reincubation at short intervals was always practiced when mixed cultures were used or when the case was found to be resistant.

\*Since the above was written he has had a recurrence of the acne and has taken other treatment for it under a skin specialist.

## CASE REPORTS

CASE 1—C S (Long Hospital, 23262), male, aged fifty five. This was the first case treated, and as a result of inexperience not as well handled as have been subsequent cases. This patient was selected because his condition was hopeless from the first, and for the reason we were more inclined to try the new method of treatment. His primary injury was a crushed pelvis resulting from an accident in which a tree fell across his body. The bladder was ruptured and there was extravasated urine throughout the pelvis. Large amounts of foul pus containing *Bacillus coli* were pouring from various sinuses, and from the bladder. Several weeks had passed since the accident and the patient was at the point of death when treatment was begun. Almost his entire back was covered with bed sores. There was an immediate change in the nature and the amount of the pus, the odor disappeared almost entirely, and after a few days there was evidence of new granulation tissue, the openings of the sinuses seemed more healthy. The man died as a result of the extension of the bed sore to the spinal canal. Autopsy showed a healthy condition of much of the tissue lining the various abscess cavities of the pelvis.

CASE 2—Chad (Riley Hospital, 3225), female, aged five. The child had had mastoiditis. For six weeks there had been no hope of recovery, the temperature was about 104 to 105°, she was comatose, and large amounts of extremely offensive pus were pouring out. Diagnosis of brain abscess had been made. *Staphylococcus aureus* and *Bacillus pyocyaneus* were isolated. Active bacteriophage action was obtained against both organisms, we had been working on the development of an active bacteriophage for this case for several days before being given the opportunity to use it. Her condition appeared immediately to improve after the filtrate was used, the temperature went down, odor and pus disappeared entirely, coma was relieved so that the child could talk and ask questions. The marked improvement continued for four days when there was sudden relapse and later death. Autopsy revealed a cerebral abscess involving nearly the entire left hemisphere, and an extreme suppurative pansinusitis.

CASE 8—Bur (Riley Hospital, 4012), male, aged sixteen, was thought to have an unknown focus of infection which caused him to develop numerous large abscesses in various parts of the body at intervals of a week or less. When opened these abscesses would drain for weeks before healing. *Staphylococcus aureus* was isolated from the pus. Active bacteriophage was obtained and injected into two unopened abscesses, one in the left breast and the other in the left thigh. A similar lesion in the right breast had drained for three weeks after being opened, while the one injected with bacteriophage was dry from the first after being opened, and it healed much more quickly. The abscess in the thigh healed much more promptly than had been anticipated, and no other lesions developed. The boy was discharged well.

CASE 31—R S (St Francis Hospital), male, aged thirty, had a discharging appendiceal abscess that would not close. It had been dressed daily for three weeks, and there was still a considerable amount of pus. *Staphylococcus aureus* was the organism. The pus stopped, and prompt healing followed the use of the filtrate prepared for another case.

CASE 40—H (St Francis Hospital). Following a nephrectomy operation the wound became infected with *Staphylococcus aureus*, and there was much pus. After a few days of treatment with the bacteriophage the wound healed completely.

CASE 34—Mrs H (St Francis Hospital), aged thirty seven. This patient, who had been brought into the hospital six weeks previously in a delirious condition, had an abscess between the skin and the sternum that was eleven inches across, and from this there poured 250 to 350 cc of putrid pus daily, she also had a left pyosalpinx, marked pyelitis, cystitis, pus, blood, and casts in the urine in large amounts, a badly degenerated myocardium, hemoglobin of 40 per cent, and red cell less than 2,000,000, in spite of three rather recent transfusions, and was running a septic temperature.

An unusually active bacteriophage was obtained against strains of *Bacillus coli* and *staphylococcus* isolated and reisolated from the abscess. The odor of the pus was mostly gone after one day's treatment, the abscess was dry after a week, and her general condition was much improved, the mental condition improved so that the patient became rational the temperature fell almost at once to 100 to 100.6 and she began to eat better and to take interest. About this time an abscess developed on the right arm. Bacteriophage was injected into it before it was opened, three injections of about 2 cc each. An incision was then made (one and a quarter inches long) and an ounce and a half of very liquid pus was evacuated. After the first drainage there was never any pus from this abscess and five days later the incision was completely healed and the abscess cavity closed without redness, soreness or induration, and this in spite of the fact that three clean transfusion wounds were still open after weeks of time. The filtrate was subsequently applied to these wounds and they healed in a few days. The large abscess over the sacrum about this time began to close in quite rapidly. One month after beginning the treatment this abscess was about one third its former size, and the patient was gaining weight at the rate of nearly a half pound a day. Her blood condition was much improved in spite of the fact that there were no more transfusions. Twenty one days after beginning the treatment she was afebrile and remained so. Ninety days after entering the hospital, and forty five days after the bacteriophage was first used she went home well. Several good physicians had considered recovery impossible. This is a conservative statement of a case that can only be appreciated by having been seen.

CASE 2—F. D. (Ruley Hospital, 3471), female aged five had a subphrenic abscess which had been drained but was not closing properly. Large amounts of thick pus continued to drain. Specific bacteriophage was instilled into the cavity and the pus immediately decreased. The bacteriophage was discontinued after a few days and the pus returned. It was then resumed, the pus stopped and the cavity closed rather rapidly. The child was sent home well.

**Ulcers**—Ulcers of various sorts have been treated very much as have been abscesses. The most surprising results have been those in connection with the treatment of bed sores. We had supposed that these were atrophic ulcers due to pressure and devitalization of tissue rather than bacteria, but it should be remembered that there is always infection in them, the commonest significant organisms being *Bacillus coli* and *staphylococcus*. The fact that these cases may be improved by this treatment was discovered quite accidentally when it was noticed that the bed sores of the first case treated began to improve when the filtrate was instilled into the bladder in the treatment of the coincident cystitis. It was quite surprising to see large bed sores become clean and start to heal, to see the pus checked, and to find that the odor had gone. So far as we can find this is the only reference to the treatment of bed sores with bacteriophage in medical literature.

#### CASE REPORTS

CASE 5—A. C. (Long Hospital, 24234), male aged sixty nine, had had hypertrophy of the prostate, residual urine, severe cystitis. A suprapubic cystotomy had been done before coming to the hospital. A periurethral abscess had developed, he was running a septic temperature and was very thin and weak. Two large bed sores developed. One over the sacrum was about six inches in diameter and was deep. The other on the leg was about three by five inches. His condition was such that death seemed imminent. Bacteriophage dressings of the sores were begun, and the same material instilled into the bladder. The temperature fell to normal the next day and remained there. The bed sores began to



clean themselves, and were dry and odorless at the end of three days. At the end of a week there were healthy granulations, and new epithelium was spreading from the edges. After a month the bed sores were practically healed, the cystitis was much better, and the surgeon was ready to remove the prostate. The patient, however, refused further surgery and went home in good condition.

CASE 13—K B (City Hospital) was a colored boy, aged fifteen, with a complete transverse ascending lesion of the cord. There was complete sensory and motor paralysis from the hips down, and there was incontinence of bladder and bowel. The trouble was of several months' duration and the legs were covered with large and small bed sores which were extremely foul. There was of course no hope of curing the primary condition, but it was thought that we might be able to control the pus and odor thereby making the case easier to handle. A fairly active bacteriophage for mixed cultures of *Bacillus coli*, *Staphylococcus aureus*, and *Bacillus pyocyaneus* was obtained. Immediately after the treatment began the odor stopped almost entirely, the sores became clean and healthy in appearance. After a week there was evidence of healthy granulation tissue. The boy complained that the ulcers next to the sensitive skin were itching and smarting, interpreted as an evidence of healing. Five weeks after the treatment began the patient died due to the progress of the primary lesion causing an ascending paralysis. At the time of his death there were ulcers two inches in diameter which had completely healed and most of the other larger sores had a rim of about one inch of new epithelium about them, were clean, and had good granulations in the base.

CASE 27—I P (Long Hospital, 25280), male, aged eighty five, had had a cancer of the upper lip which had been removed surgically and also treated with radium. There was an ugly pus filled ulcer on the upper lip. Local applications of an autogenous anti-staphylococcus bacteriophage filtrate caused relief of pain and soreness, and soon cleaned the ulcer. It healed in a few days except for one place about one centimeter in diameter, which was considered to be a radium burn. The patient after several weeks developed erysipelas. This condition was improving when pneumonia set in and the patient died. Autopsy examination revealed the fact that the ulcer had at last entirely healed.

CASE 37—T (Long Hospital, 25632), male, aged fifty six, had had a cancer of the lip removed months before. Metastasis had involved a neighboring lymph gland, and this was removed with the cautery knife leaving a denuded area about eight by ten centimeters. This wound became infected with *Staphylococcus aureus* and made a rather severe sore. To answer the criticism of a colleague, simple broth of the kind that we used for the making of the bacteriophage was used at first. There was an increase in the amount of pus under this treatment after a week. Bacteriophage filtrate was then used and the denuded surface became clean in twenty four hours. After a few days there was healthy granulation tissue over the entire surface. The patient was sent home at this time. Three weeks later he returned with the wound doing well and about half its former size.

*Acne*—Early in our experience with the therapeutic use of bacteriophage we noticed a student with an extremely bad case of pustular acne. We suggested trying the use of bacteriophage, but with no great assurance of being able to give help. This is the first case reviewed below. Our results in the treatment of acne have been peculiar in that we have had better success in the bad than in the mild cases. As soon as the active inflammatory stage of the condition has been cleared we have usually recommended x-ray in the hope that the oil glands of the skin may be permanently checked in their excessive activity, thereby removing the principal cause of the condition. The organism isolated has in nearly every case been *Staphylococcus albus*, rarely

aureus. The attempt has not been made to isolate the "acne bacillus" as we have been inclined to the belief that it is only a secondary invader and not the etiologic agent.

#### CASE REPORTS

CASE 9—B, a medical student aged twenty years. This was a severe case of pustular acne. The lesions were not deep and there was no permanent scarring. There were about thirty five active pustules on the face when we began. The face was sore, indurated and beefy red. Active bacteriophage action was obtained against the organism. The filtrate was made into a soft agar which was to be rubbed into the face twice a day after opening the pustules, then cleaning the face with alcohol, and finally washing with strong soap and hot water. In as much as this preliminary treatment had been used energetically for years without results it cannot be said that this was the cause of the improvement. There was a marked change in the space of three days. After three weeks there was scarcely an active lesion, and after six weeks the skin was soft and smooth and has remained so.

CASE 10—C, J, male, aged eighteen, had a bad case of acne with deep pits and much induration. He had lost his job because of the condition of his face and could find no other employment for the same reason. His acne was of four years' duration and was getting worse and had resisted every sort of treatment including x-ray. Treatment as in the case above was instituted. At the time that we began his face was red, sore, and hard. Very little pus could be expressed from the lesions. After a week the face was much softer and about three cubic centimeters of pus was expressed from the various lesions. A week still later the face was even softer, but very little pus could be obtained. After this there was little evidence of active inflammation though the face was of course badly scarred. His appearance was however greatly improved and he was able to get a job. Since this time he has been unable to come to the laboratory and we have not been able to keep in contact with the case.

CASE 11—P. K. L., a medical student aged twenty one had had severe acne for years. His face was badly scarred, pitted, indurated, and quite sore. Treatment as above described gave excellent results. The face became much softer, and it was possible to get a large amount of inspissated pus from the old lesions deep in the skin. No new pustules have developed since the treatment was begun. The pits and some of the nodules have remained. He has had several x-ray treatments since stopping our treatment and is now much improved in appearance.

CASE 8—Bur (Riley Hospital, 4012). The healing of abscesses in this case has already been described. The patient also had a marked pustular acne which entirely disappeared in the space of three weeks after we began using on his face some of the same filtrate that was used for the abscesses.

CASE 12—M. F., male, aged nineteen, a strong athletic type with a marked pustular acne on chin, lips, and nose. There is no scarring. Relief came slowly, but finally all lesions were healed under the bacteriophage treatment. The use of the agar containing the active principles was then discontinued and the pimples began to reappear. Treatment was resumed and they again disappeared. X-ray was recommended to check the excessive oiliness of the skin.

CASE 16—G. K., female, aged eighteen. This was a mild case. After two months the face is clear, but the case was so mild that the result is inconclusive.

CASE 17—K. L., male, aged seventeen. This was a moderate case in a youth in excellent health. The case is still under treatment. Progress indicates that we may expect good results, but that it will be slow.

CASE 19—J H, male, aged twenty This is a moderate case of six months' duration The youth is away at college, and we have not seen him recently, but good reports have been obtained

CASE 22—Card (Long Hospital, 25582), male, aged twenty one This was a rather marked case in a patient in the hospital for an appendectomy He was sent home eight days after we began treatment, and at that time was well of acne except for the red blotches where the pustules had been No new ones had developed

CASE 25—F F, male, aged twenty three This was a moderate case with scarring There is marked improvement At one time three boils complicated the picture Staphylococcus aureus was obtained from these boils When bacteriophage active for both Staphylococcus aureus and albus was used we got excellent results

CASE 26—M B, a young woman with very slightly active lesions, but considerable scarring She insisted on using bacteriophage though we told her it would probably do no good in her case She thinks there is less induration, and active inflammation since using the preparation, but of this we cannot be sure

CASE 33—Ne, a student nurse with a moderate case of acne is still under treatment with little improvement Immediately after the bacteriophage was first used there was apparent help, but as soon as she stops using it the pimples return about as they were at first

CASE 38—F N This was a severe case in a man of twenty two He was very recently discharged completely cured of active lesions His skin is excessively oily and x-ray is recommended

CASE 44—C F This was a severe case in a boy of sixteen He was much improved after the first week's treatment, and has not been seen since

*Urinary Infections*—Among the first attempts made to use bacteriophage therapeutically, were several cases of urinary infections due to *Bacillus coli* We have treated no patients for cystitis alone, but in several of our other cases we have seen good effects come from its use The filtrate can be instilled into the bladder as there seems to be no bad effects from such treatment We have not seen the extremely rapid results reported by certain authorities, but have not failed to observe marked improvement in every case in which an active bacteriophage has been injected into the bladder of patients with *Bacillus coli* infection

Cases 1, 5, and 34 are instructive in this connection An interesting incident in connection with a case for which we were preparing an autogenous bacteriophage should be recounted here We were ready to send the material when the physician in charge notified us that it was not necessary as the woman had quite suddenly greatly improved, and was practically well of her cystitis We asked for a sample of her urine and found that it contained a strain of bacteriophage very active for the strain of *Bacillus coli* previously isolated from her urine In this case the woman had evidently developed her own bacteriophage, and this was, we believe, responsible for the rapid change in her condition This woman had other relapses following this, however, and also other rapid improvement periods We have been unable to study her urine since as she lives at a distance from the laboratory

Sewage is an excellent source of highly active *Bacillus coli* bacteriophage and little difficulty is experienced in preparing it from this source Those who wish experience with this most interesting phenomenon may well begin with sewage as a source of *Bacillus coli* bacteriophage

## MISCELLANEOUS CASES

CASE 18—S D, a physician, aged fifty nine, with inoperable cancer of the bladder of long standing. The growth had obstructed the return circulation of the penis and as a result a painful balanitis had developed requiring the use of over a grain of morphine daily to make life tolerable. There was imminent danger of moist gangrene. A pure culture of *Staphylococcus aureus* was obtained and an active bacteriophage filtrate prepared. There was prompt relief from pain and complete healing of the balanitis in about a week in spite of the fact that the primary condition was progressively becoming worse. At the time the treatment was begun the balanitis was of eight months' duration and becoming worse. The treatment was stopped, and the ulcers returned after about three weeks.

CASE 32—H (Long Hospital, 2249) female aged twenty two. About a year before this case was admitted to the hospital she had had an operation for tuberculous peritonitis and had been left with a fecal fistula. At the time that we began the treatment there was a large ulcerated area (12 by 15 cm) about the mouth of the fistula. The edges were deeply undermined in several places as much as ten centimeters in one direction. Fecal matter in large amounts came into the wound constantly. There were large amounts of pus and it was necessary to dress the wound three times a day. The patient was getting worse, was extremely emaciated and subject to delirious attacks warranting the diagnosis of toxic psychosis. When it was decided to send her home to die, we began to treat the infected sinus with bacteriophage and she was retained in the hospital for this special treatment. The amount of pus was soon reduced, the odor was less offensive, the wound became cleaner and good granulation tissue developed at the base and sides. The opening in the skin is much smaller than it was when we began the undermining as now no more than 2.5 cm at the deepest place and her general condition is better. At one time there had been no feces in the wound for a month when a mistake in the use of ultra violet light (probably) greatly stimulated her causing her to become violently delirious, and causing her to tear the fistula open again. Feces again came into the wound, but has not been seen in the last two weeks. No attempt has been made to treat the tuberculosis except that good general care has been given and intravenous glucose has been used about twice a week much of the time. Light was used twice and seemed to make her worse. The case has been under treatment for twelve weeks and is still a very grave one. There can be no doubt that there has been marked improvement in the wound and in the patient's general condition during this time, and this in spite of the fact that she had been going down hill for a year under all other methods of therapy.

CASE 24—Inf R (St Francis Hospital) female, aged fourteen days. On the tenth day of life this child developed impetigo over a considerable portion of the body. Anti-staphylococcus bacteriophage was used on a part of the lesions while the others were dressed with ammoniated mercury ointment. Two days later all the lesions were improved but those treated with bacteriophage were more improved. This treatment was then used on all of the lesions and they were completely well in two more days. This result was better than had been commonly attained in such cases in that hospital.

CASE 4—M S (Riley Hospital, 3567), female aged seven. This child had an osteomyelitis of the os calcis. The bone had been removed but the entire foot was at the point of becoming gangrenous. Amputation was seriously considered. There was prompt improvement and recovery when the filtrate was used as an irrigation and a wet dressing.

CASE 49—P B (Long Hospital, 25857), male aged twenty six. This patient had had a discharging sinus in the region of the left anterior superior spine of the ilium for seven years. The bone was thought to be involved but exactly in what manner was unknown. There was immediate relief from pain, tenderness, and soreness when the irrigations were begun, and the patient was sent home well after about three weeks' treatment.

## SUMMARY

1 Bacteriophage filtrates active for autogenous cultures have been used in a series of fifty suppurative conditions and have been found to be highly effective against *Staphylococcus aureus* and *albus*, *Bacillus coli*, and *Bacillus pyocyaneus*

2 They have been found most effective when used as a wet dressing or when instilled into a cavity

3 There is considerable evidence that stock cultures of the bacteriophage may be used with profit when there is not time or facility for the preparation of the autogenous product, or while it is in preparation. This is particularly true of the *staphylococcus* preparations

4 Those who have seen the results in actual cases are invariably enthusiastic and are convinced that the method has much merit. At least it offers promise and should be thoroughly investigated

## REFERENCES

- 1D'Herelle The Bacteriophage and Its Behavior, Williams and Wilkins, 1927
- 2Dutton Jour Infect Dis, 1926, *xxix*, 48
- 3Shartzman Jour Exper Med, 1927, *xl*, 497
- 4Besredka Local Immunization, Williams and Wilkins, 1927
- 5Bourdenko Ann d l'Inst Pasteur, 1926, *xl*, 232
- 6Carrere Ann de l'Inst Pasteur, 1925, *xxxix*, 67
- 7Epstein Wien klin Wchnschr, 1927, *xl*, 80
- 8Ibid Wien klin Wchnschr, 1927, *xl*, 601
- 9Gloukhoff Ann de l'Inst Pasteur, 1927, *xl*, 189
- 10Lehndorff und Brumlik Wien klin Wchnschr, 1927, *xl*, 483
- 11Nicolas Ann de l'Inst Pasteur, 1926, *xl*, 1073
- 12Nikolaewi Ann de l'Inst Pasteur, *xl*, 1926, 869
- 13Hayes Laryngoscope, 1926, *xxxvi*, 812
- 14Arnold und Weiss Jour Lab and Clin Med, 1926, *xii*, 20
- 15Bazy Compt rend Soc de biol, 1925, *xen*, 485 (Abst Jour Am Med Assn, *lxxxix*, 1389)
- 16Rice Jour Indiana Med Assn, 1928, *xli*, 93
- 16aFriedlander and Toomy Jour Exper Med, 1925, *xlii*, 663
- 17Besredka Ars Medici, 1927, *v*, 511
- 18Hauduroy, Cimus und Dalsace Presse med, September 22, 1926
- 19Besredka Local Immunization, 1927, Chapter II
- 20Bruynoghe und Musin Compt rend Soc de biol, 1926, *lxxxv*, 1120
- 21Callow Jour Infect Dis, 1927, *xli*, 124
- 22Gratia Compt rend Soc de biol, 1924, *lxxxvi*, 276
- 23Becherich und Handuroy Jour Bacteriol, 1923, *viii*, 163
- 24Larkum Jour Michigan Med Soc, 1928, *xxvii*, 106
- 25Gougerot und Peyre Compt rend Soc de biol, July 12, 1924
- 26Nelson Jour Immunol, 1928, *xi*, 43
- 27McKinley Jour Lab and Clin Med, 1923, *viii*, 311
- 28Ibid Arch Int Med, 1923, *xxxii*, 899
- 29Dalsace Jour d'Urol, 1926, *xxi*, 123
- 30Frisch Wien klin Wchnschr, 1925, *xxviii*, 839 (Abst Jour Am Med Assn, *lxxxix*, 861)
- 31Munter and Boenheim Ztschr f Kinderh, 1926, *xxxix*, 388 (Abst Jour Am Med Assn, *lxxxv*, 78)
- 32Larkum Jour Bacteriol, 1925, *xii*, 223
- 33Ibid Jour Bacteriol, 1926, *xiii*, 203-240
- 34Zdansky Wien Arch f inn Med, 1925, *xi*, 533 (Abst Jour Am Med Assn, *lxxxvi*, 317)
- 35Ravini Presse Med, May 1, 1926, 548

# SOME COLOR REACTIONS OF MORPHINE AND ITS DERIVATIVES ON HEATING IN CONCENTRATED SULPHURIC ACID SOLUTION\*

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## INTRODUCTORY REMARKS

THE present paper, though complete in itself is a continuation of the previous one entitled "Some New and Improved Tests for Morphine and Related Alkaloids." In that paper among other things, there were described some reactions obtainable after heating morphine, or one of its derivatives, in pure concentrated sulphuric acid to various temperatures below that at which a color develops in the sulphuric acid solution itself, the temperature at which the sulphuric acid color test begins. The more important points in this connection are mentioned in article VI of the present paper. Name of the Reactions."

No attempt is here made to discuss the reactions of any alkaloids other than morphine, heroine, codeine, and diionine. The investigation of these tests requires pure substances, for with heating in concentrated sulphuric acid other organic matter if present might char and spoil the reaction. For some of the tests, the alkaloid must also be free from chloride or bromide. Time and opportunity have been lacking, thus far, for the trial of many alkaloids besides those mentioned. But indications are that all these tests are specific for morphine, and its most closely related derivatives. Substances giving similar reactions, if any exist, are no doubt phenols rather than nonphenolic alkaloids.

The exact amount of morphine required for a test is not stated in the descriptions. About as much should be taken as is ordinarily used in any well known test of the same general character. For instance, Pellagri's test. References to "large" or "small" amounts of morphine are to be interpreted with this in mind.

I have attempted to make the descriptions brief and at the same time mention all effects of any importance. In general amyl alcohol, chloroform ether, and benzol have been tried as solvents for each colored product, not only for extraction from the dilute sulphuric acid solution, but also from solutions to which ammonium acetate sodium carbonate or bicarbonate, and ammonia had been added. It may be mentioned here that amyl alcohol will always extract the color if anything will.

It is not possible to give the temperature for a change with great exactness, because a change which occurs almost immediately at a certain temperature will take place gradually at lower temperatures. In the descriptions the temperatures stated were obtained, as a rule, by placing the test tubes in the

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bath at about room temperature, and heating gradually, but fairly rapidly, until the desired temperature was reached. A different method is to be followed only when it is stated that the test tube may be stood in the boiling water-bath for a certain length of time.

Objection may be made to the names I have assigned to these tests. Presumably it is actually arsenic acid which takes part in the arsenate test and chromic acid in the chromate test. However the name "arsenate test" seems to be well established, so I have adhered to it, and named the other tests to correspond. In the "halide-oxidation test" it is solid chloride or bromide (of sodium) that is added, but apparently free chlorine or bromine that actually reacts with the morphine.

None of these tests should displace any from a list of the 10 or 12 best tests for morphine, but they are not without value, as well as interest. The arsenate test, regulated by the thermometer and properly understood, is an excellent qualitative test and the same may be said of the perchlorate test. The new halide-oxidation test should prove even more useful.

#### MATERIALS USED

The alkaloids used in investigating these tests, with the source of each, were as follows:

- Morphine hydrochloride—from samples
- Morphine sulphate—Mallinckrodt
- Morphine (free alkaloid)—prepared from the hydrochloride by precipitation with ammonia
- Codeine sulphate—Mallinckrodt
- Codeine (free alkaloid)—N. Y. Quinno and Chemical Works
- Heroin hydrochloride—Mallinckrodt
- Heroin (free alkaloid)—prepared from the hydrochloride by precipitation with ammonia
- Dionine (hydrochloride)—Merck
- Ethyl morphine hydrochloride—Powers Weightman Rosengarten Co.
- Dionine (free alkaloid)—prepared from the Ethylmorphine hydrochloride (P. W. R. Co.) by precipitation with ammonia

The sulphuric acid used was the C. P. acid, 95 per cent  $\text{H}_2\text{SO}_4$ , manufactured by the General Chemical Company. The analysis of this acid, as given on the label, was included in my previous article, referred to above. The U. S. P. sulphuric acid manufactured by the Hirsch Laboratories was also tried in some tests and gave exactly the same results.

#### I. THE SULPHURIC ACID COLOR TEST

When morphine is dissolved in pure concentrated sulphuric acid, and the solution heated, little or no color is developed for some time. In fact, the only color appearing below  $130^\circ$  or  $140^\circ \text{C}$ , if the acid and alkaloid are both pure, will be a slight violaceous or reddish color, doubtless due to a little oxidation by atmospheric oxygen. At about  $140^\circ$  or  $150^\circ \text{C}$ , however, with very little morphine, a dark olive-green color develops.

That morphine gives a color test in this way is, of course, well known. The color on dilution and on addition of ammonia have also been noted, but the colors extracted by amyl alcohol seem to have escaped attention hitherto. The test is as follows:

Put a little dry morphine or its salt in a clean, dry test tube, and dissolve it in a little pure concentrated  $H_2SO_4$ . Stand the tube in a suitable bath (say of phosphoric acid), provided with a thermometer, and heat it about 140 to 150 C. a dark olive green color develops. Continue heating to about 175 C. The solution is then green black. Cool, dilute with a few c.c. of water. The aqueous solution is dark blue green, and a dark precipitate falls if it is allowed to stand. Divide this solution into two portions. To one portion add about 0.7 c.c. amyl alcohol and shake. The amyl alcohol readily extracts a deep slightly purplish, blue color (ether, chloroform, and benzol extract little or no color). To the other portion add concentrated  $NH_4OH$  until alkaline. It becomes clear green. At once add a little amyl alcohol and shake. It extracts a bright green color. The color remaining in the aqueous layer soon changes from green to brown, and the green in the amyl alcohol also changes to brown more slowly.

Heroin, codeine, and diacetylmorphine give this same reaction. Even in the presence of impurities which clear and spoil the other colors it is possible, some times at least, to get good blue and green colors in the amyl alcohol extracts.

If the sulphuric acid solution is cooled and diluted after heating only to 160° C., amyl alcohol extracts a purple rather than a deep blue color. The other colors are about the same, but not so strong as when the heating has been carried to 175°.

The colors obtained in this test should be compared with those obtained in the arsenate and chromate tests at similar temperatures.

## II. THE ARSENATE TEST

Several authorities, for instance Allen's 'Commercial Organic Analysis,' and Fresenius Wells' 'Qualitative Chemical Analysis,' describe a color test given by morphine on heating in sulphuric acid containing a little sodium or potassium arsenate. These descriptions, without exception so far as I have found, are incomplete, fail to recognize the influence of chloride if present, state no temperatures, and in short are so misleading as to be almost worse than useless. They usually say also that codeine gives the same or a similar test.

The colors developed in the sulphuric acid solution constitute a test, and if we consider separately the colors extracted by organic solvents, then there are at least two other distinct tests in the arsenate reaction, depending on the temperatures to which the solutions are heated. Heating to 130° C. provides one set of colors in solvents, heating to 175° an entirely different one. Chloride which may be present as morphine hydrochloride, is capable of changing the reaction completely. Bromide changes it even more readily. With codeine, the color changes in the sulphuric acid solution are entirely different from those produced by morphine.

*Morphine*—First Arsenate Test. Color changes in the sulphuric acid solution.

Put a little morphine in the form of the free alkaloid or the sulphate, in a clean dry test tube, and dissolve it in a little pure concentrated  $H_2SO_4$  (say about 8 drops). Add 4 drops of concentrated  $H_2SO_4$ , containing 5 gm.  $KH_2AsO_4$  per 100 c.c. (Not much arsenate is required for a strong reaction, but, for temperatures below 150 C. or thereabouts, an excess does no harm.) When the arsenate is added as soon as the morphine is dissolved, a blue color develops at once if a fair amount of morphine is used. When very little morphine is used this blue color will not be noticed, as it is not strong. Likewise if the morphine has stood for some minutes in the concentrated  $H_2SO_4$  solution before adding the arsenate, this blue color is likely to be missed. Stand the test tube in a suitable bath (say of phos



phoric acid), and heat (This is much better than trying to heat it gradually and steadily over a free flame) On heating to 30 to 40°C, or on standing for some minutes at room temperature, the color changes to green (or, if there has been no blue, a green color now develops) On continuing the heating, the color soon becomes dark green, at 60 to 70° it begins to change to blue, and soon becomes dark blue, at about 115° it changes back to dark green This color, or green black, persists until about 200°, when charring makes the solution a dirty brown black

**Second Arsenate Test** Colors extracted by organic solvents after heating to 130°C

Dissolve morphine in sulphuric acid, add arsenate, and heat, as described in the "First Arsenate Test," above Heat to 120 to 145°C, preferably about 130°, then remove the test tube from the bath, cool, and dilute with about 6 cc water A dark blue solution is formed from which a dark precipitate soon falls on standing Divide into 3 or 4 portions, and shake each with an organic solvent Amyl alcohol or chloroform extracts deep indigo or purple, ether crimson, and benzol deep violet red Nearly all the color is extracted from the aqueous layer in each case, ether, however, not being quite as good a solvent as the other three

**Third Arsenate Test** Colors extracted by organic solvents after heating to 175°C

Heat morphine in sulphuric acid solution, with arsenate, as described above, but to 170 to 180°C, preferably 175° (Use only 1 or 2 drops of the arsenate solution if the amount of morphine tested is quite small) Then cool and dilute with about 6 cc water A blue green black solution is obtained, with a dark precipitate on standing Amyl alcohol extracts deep (greenish) blue, chloroform green blue, ether and benzol blue Generally, considerable color remains in the aqueous layer, and chloroform or benzol may form an emulsion which prevents the color extracted from being seen readily If a portion of the solution is made alkaline with concentrated  $\text{NH}_4\text{OH}$ , it turns a clear bright green, and from this solution amyl alcohol extracts green These greens do not turn to brown as in the sulphuric acid color test In this "Third Arsenate Test," but not in the two preceding, heroine, codeine, and dionine all give the same results as morphine

**Dilution below 115°C**

If the morphine arsenate solution is heated only to 105 to 110° (just before it turns from blue to green), and then diluted, it gives a green or olive green solution in which a dark precipitate forms on standing, and from which solvents extract the same colors as after heating to 130°, but much weaker

If the dilution is made after heating from 90 to 95° (when the concentrated acid solution is strongly dark blue), a yellowish olive brown or olive green solution is obtained, from which amyl alcohol will extract a little blue Addition of ammonia to a portion of the aqueous solution makes it brownish red, and from this solution amyl alcohol extracts a red color

If the dilution is made at 50 to 55° (when the concentrated acid solution is a strong green), a light yellow or orange yellow solution is obtained, which cannot be depended on to yield a color to organic solvents

**Use of morphine hydrochloride**—This test has been restricted, in the description above, to the free alkaloid or the sulphate The use of the hydrochloride of morphine tends to produce the effects of the halide oxidation test However, morphine hydrochloride may be used for the arsenate test as follows Take no more than a moderate amount, dissolve in pure concentrated  $\text{H}_2\text{SO}_4$ , shake the tube and let stand a minute or so for the  $\text{HCl}$  gas to escape Then add the arsenate Color variable at first, blue, purple, red or brown, according to the extent of interference with the normal test Heat, and the subsequent succession of colors should be as already described at 30° becoming green, at 65° blue, at 115° dark green On cooling and diluting after heating to 130° or 175°, the same results will be obtained as have already been described for the second and third arsenate tests If dilution is made below 100°, it will be found that amyl alcohol will readily extract a blue green color Some other chloride effects may be found superimposed on the arsenate reaction, at these low temperatures

**Heroin**—Heroin gives the same reaction as morphine. Both for legitimate and illegitimate purposes it is commonly sold as the hydrochloride therefore see the remarks above concerning the use of morphine hydrochloride for the arsenate test.

**Codeine**—First Arsenate Test. Color changes in the sulphuric acid solution.

Codeine does not develop a color in the arsenate test so readily as morphine does. Unless the amount taken is rather large there is no color until about 75 to 80 C is reached and in any case there is at first simply a gradually deepening purple color, and no actual change of color until about 115 to 120 C is reached when the solution becomes dark green or green black as with morphine. The green color persists, as with morphine until charring destroys it.

**Second Arsenate Test** Colors extracted by organic solvents after heating to 130 C.

In this test the same colors are obtained with codeine as with morphine but the solvents extract their colors much less successfully leaving the aqueous layer decidedly blue. This is especially noticeable with ether which extracts no more than a pink leaving the aqueous layer dark blue, while with morphine if shaken thoroughly, ether extracts practically all the color, becoming deep crimson.

**Third Arsenate Test** Colors extracted by organic solvents after heating to 175 C.

In this test codeine reacts substantially the same as morphine.

**Dilution below 115 C**

Diluted at 90, when the concentrated acid solution is purple, codeine gives a solution but slightly yellowish, from which solvents do not extract any noticeable color. The similarity to morphine begins at about 100 the results on diluting at 105, as at 130 being like those of morphine but weaker.

**Use of Codeine hydrochloride**—Fortunately the hydrochloride of codeine is a salt seldom met with. The presence of chloride will tend to cause the same reaction with codeine as with morphine since the chloride oxidation test is the same for the two alkaloids. However, if practically all of the HCl gas is allowed to escape before adding the arsenate the color in the concentrated sulphuric acid solution (particularly at 50 to 60 where morphine gives green) will be sufficiently different to distinguish the two alkaloids easily by means of this test.

**Dionine**—The dionine reaction is naturally very like that of codeine, but there are differences due to the fact that the ethylmorphine compound breaks down more readily than the methylmorphine compound. Dionine develops a purple color more readily than codeine usually at about 50 C, or sooner. Dionine begins to react like morphine at about 75, instead of 100 as for codeine. By the time dionine has been heated in concentrated sulphuric acid to 100 C, its reactions are practically identical with those of morphine after the same treatment. Dionine, therefore, does not show those differences from the morphine reaction which distinguish codeine, after heating to 130. As dionine is commonly sold in the form of the hydrochloride caution must be used in distinguishing it by means of the arsenate test. See the remarks concerning the Use of codeine hydrochloride, above.

COMPARISON OF THE SULPHURIC ACID COLOR TEST WITH THE THIRD ARSENATE TEST

	MORPHINE HEATED IN PURE CONC H <sub>2</sub> SO <sub>4</sub> TO 175 C	MORPHINE HEATED IN H <sub>2</sub> SO <sub>4</sub> -- ARSENATE SOL. TO 175 C
Concentrated acid solution	Green black	Green black
Diluted with water	Dark blue green	Dark blue green
Amyl alcohol extracts	Deep blue (purplish)	Deep blue (greenish)
Ether extracts	No color or faint blue	Good blue
Chloroform extracts	Scarcely any color slight green	Deep blue green
Ammoniacal solution	Clear green changing on standing to olive then brown	Clear green
Amyl alcohol extracts from NH <sub>4</sub> OH sol	Bright green (from green sol) on standing overnight both layers brown	Bright green on standing over night both layers still green

## III-A THE CHROMATE TEST

"Potassium dichromate (added to the solution of solid morphine in cold concentrated sulphuric acid) is reduced with the production of a green color"—Allen's Commercial Organic Analysis

This is a very misleading statement, as the color is due to the oxidized morphine, rather than to the reduced chromium. In fact, chromate is only a little less effective than arsenate in producing various colored compounds from morphine. There is this important practical difference, however, that chromate is so much more active an oxidizing agent that it must not be present in excess, otherwise the colored oxidation products will be destroyed on heating. Usually, therefore, the maximum effect of the total amount of morphine taken cannot be obtained, and so the chromate test, though of considerable interest, has no very great practical value.

Morphine, heroin, codeine, and diomine give substantially identical effects in the chromate test.

*First Chromate Test*—If a little morphine is dissolved in concentrated sulphuric acid on a spot plate, and a small crystal of dichromate crushed and stirred in (as for the "Fading Purple Test" for strychnine), the solution becomes first brown, and then gradually brownish-green. If this solution is poured into a test tube, and diluted with about 2 cc of water, it gives an orange or reddish-orange solution, and on shaking with a little amyl alcohol the latter becomes bright green or bluish green. Thus morphine may be detected by means of the test for strychnine, but in testing for morphine specifically it is probably better to use the "Second Chromate Test" or to proceed as follows for the "First Chromate Test"

Put a little morphine in the form of the free alkaloid or the sulphate, in a clean dry test tube, and add (say) 8 drops pure concentrated  $\text{H}_2\text{SO}_4$ , or enough to dissolve the morphine readily. When it is dissolved, add 4 to 6 drops  $\text{H}_2\text{SO}_4$  containing 0.2 gm  $\text{Na}_2\text{Cr}_2\text{O}_7$  per 100 cc. This addition must be made in the cold, even though the solution is heated immediately after. In a very warm room it will be necessary to cool the acids beforehand. Shake the tube slightly to mix the solutions, then warm gently to  $45^\circ$  to  $50^\circ\text{C}$ , not above  $60^\circ$ . The reaction takes place rather gradually at room temperature, warming ensures oxidation as complete as the small amount of chromate permits. For mere traces of morphine the amount of chromate must be reduced. With a speck of morphine dissolved in 4 or 5 drops of  $\text{H}_2\text{SO}_4$ , and with the addition of but 1 drop of  $\text{H}_2\text{SO}_4$  containing 0.1 gm  $\text{Na}_2\text{Cr}_2\text{O}_7$ , I have obtained the reaction faintly. At low temperatures, even at ordinary room temperature,  $20^\circ\text{C}$ , there is little danger of the reaction being spoiled by too much chromate, but if any heat is applied, the chromate must not be in excess.

When the chromate solution is added and mixed in, the morphine solution becomes dark brown. At  $45$  to  $50^\circ$  it is dark greenish brown or brownish green. Dilute with a few cc of water. An orange or reddish orange solution is obtained, from which amyl alcohol extracts a bright green or bluish green color. Chloroform, benzol, and ether extract no color. Addition of ammonium acetate to a portion of the diluted solution changes its color to green, from this solution amyl alcohol extracts green  $\text{Na}_2\text{CO}_3$  added to the diluted solution changes the color first to green, then, with an excess, to brownish pink. Amyl alcohol extracts from this basic solution a greenish blue to green color, leaving the aqueous layer salmon pink. This color remaining in the aqueous layer may be due to a trace of iron in the reagent.

Codeine cannot be distinguished from morphine in this reaction, it gives dark green rather than green brown in the  $\text{H}_2\text{SO}_4$  solution, but this is the only difference. Heroin and diomine, of course, react like morphine and codeine.

If the hydrochloride of the alkaloid is used, the previously mentioned effects will be but little changed, but chloroform, ether, and benzol will extract colors. See the 'Halido Oxidation Test'.

**Second Chromate Test**—In the 'First Chromate Test' a colored compound is formed which is soluble in amyl alcohol but not in chloroform, benzol, or ether. If, however, the heating of the concentrated acid solution is carried above 70 C these other solvents will also extract colors. As the effects are much the same over a considerable temperature range above this transition point the simplest method of heating is to immerse the test tube in the boiling water bath for some thirty seconds or longer even up to several minutes.

Put a little dry morphine (free alkaloid or sulphate) in a clean dry test tube, dissolve in a few drops pure concentrated  $H_2SO_4$  add 4 to 6 drops  $H_2SO_4$  containing 0.2 gm  $Na_2Cr_2O_7$  per 100 c.c., and stand the tube in the boiling water bath for forty seconds or longer. The concentrated acid solution is then dark greenish brown. Remove the tube cool, and dilute with several c.c. water. A reddish or brownish orange solution is obtained. A portion of this solution shaken with a little amyl alcohol yields a blue green or green blue color to the solvent. Chloroform extracts a deep purple or indigo, benzol pink, ether very slight pink. If a considerable quantity of ammonium acetate is added to the aqueous solution it becomes green. Amyl alcohol then extracts a dark blue changing to dark blue green, chloroform a deep purple or indigo (as before), benzol a pink color (stronger than before), ether a good magenta pink. Thus the colors extracted by solvents are scarcely changed by the addition of  $NH_4Ac$  but in some cases they are greatly strengthened. To extract any marked color with ether the addition of  $NH_4Ac$  is necessary. At the other extreme, chloroform extracts about as deep a color from the solution containing highly ionized sulphuric acid as from the one containing practically unionized acetic acid.

**The Sulphuric Acid Color Test and the Chromate Test**—Since the morphine has to be in excess of the chromate, the free morphine naturally reacts with the sulphuric acid, when a temperature of 140 to 150 C is reached, as in the sulphuric acid color test. But how does the morphine react which has already been oxidized by the chromate? We find that it reacts in the same way, that is previous oxidation by chromate instead of spoiling the 'Sulphuric Acid Color Test' strengthens it with the addition of a few minor effects. The 'third' and 'fourth' chromate tests described below show the same colors as the 'Sulphuric Acid Color Test' but stronger and with the added effect of noticeable colors in other solvents than amyl alcohol.

**Third Chromate Test**—Treat a little morphine with  $H_2SO_4$  and chromate as before this time heating to 155 to 160 C. The solution is dark brown. Cool and dilute with several c.c. of water. The aqueous solution is blue. Amyl alcohol extracts a deep violet color. Other solvents do not extract nearly so much color but benzol extracts a readily noticeable lavender, ether a light pink and chloroform a slight blue or purple.

**Fourth Chromate Test**—Treat a little morphine with  $H_2SO_4$  and chromate as before, this time heating to 175 C. The solution is green black. Dilute with several c.c. of water. This gives a dark blue color. Amyl alcohol extracts purplish blue. Other solvents extract but little color. Benzol a light violet, ether a light pink, chloroform a slight blue. Addition of  $NH_4OH$  to the dilute  $H_2SO_4$  solution makes it clear yellowish green, and from this solution amyl alcohol extracts a green color.

### III B THE PERCHLORATE TEST

"If the sulphuric acid solution (of morphine) be heated on the water bath to 100° and a minute fragment of pure potassium perchlorate be added a deep brown or reddish brown coloration is produced which rapidly spreads through the liquid. The color is destroyed on dilution. L. Siebold to whom the test is due did not observe a similar reaction with any other alkaloid."—Allen's Commercial Organic Analysis. The U. S. Dispensatory and Blyth (Poisons, Their Effects and Detection) also give this test. Blyth and Allen state "The perchlorate must be free from chlorate which is ensured by heat

ing it with hydrochloric acid as long as chlorine [ $\text{ClO}_2$ ] is evolved. The salt is then washed with cold water and dried."

Blyth gives the reference *Am Jour Pharm*, 1873. Apparently the test has not been in actual use since that time. Perchlorate intended for use in modern laboratories does not need purification in the manner stated. I have tried the test both with such perchlorate, and also with that precipitated by a potassium salt from perchloric acid solution. The latter being in very small crystals dissolves more readily in the concentrated sulphuric acid, and is more convenient to use. The test actually obtained is very similar to the arsenate test.

*First Perchlorate Test*—Put a little morphine or its salt in a clean dry test tube, and dissolve it in a few drops of pure concentrated sulphuric acid. Add a very little solid potassium perchlorate, or better, 4 or 5 drops of a solution containing about 1 gm  $\text{KClO}_4$  in 100 cc pure sulphuric acid, for it is possible to destroy the reaction by too much perchlorate, and thus must be guarded against if the amount of morphine is small. Stand the test tube in a suitable bath, provided with a thermometer, and heat. At about  $60^\circ\text{C}$  a dark violet color develops. Should even a slight trace of chlorate be present the solution will, of course, become colored below  $60^\circ\text{C}$ , probably at room temperature the perchlorate must be entirely free from chlorate. Continue the heating the solution gradually becomes dark violaceous or brownish red rather than dark violet, then at about  $125^\circ\text{C}$  it rapidly changes to dark green. Heat to about  $150^\circ$ , then remove the tube from the bath, cool, and dilute with about 1 cc of water for every two drops of  $\text{H}_2\text{SO}_4$  used. The aqueous solution is a deep strong blue. Amyl alcohol readily extracts all the color, becoming deep violet or indigo. Ether and benzol are comparatively poor solvents, but extract readily noticeable pink and lavender pink colors respectively. Chloroform is a poor solvent for the color, extracting only a slight purplish.

*Second Perchlorate Test*—Proceed as in the "First Perchlorate Test," but reducing the amount of perchlorate somewhat, and carry the heating to nearly  $175^\circ\text{C}$ . The concentrated acid solution is still dark green. Dilution gives a very dark blue green with a dark precipitate soon forming. Amyl alcohol extracts deep blue or indigo, ether a good violet or indigo color, chloroform light blue (greenish), and benzol a slight purplish blue. Addition of ammonia changes the aqueous solution to green, and amyl alcohol then extracts green.

If the dark violet sulphuric acid solution is diluted without having been heated over  $100^\circ\text{C}$ , an orange yellow solution is obtained from which amyl alcohol will extract only a slight blue. If the heating has been carried above  $105^\circ\text{C}$ , but not so high as to make the concentrated acid solution green, or if heating in the boiling water bath has been continued for some twenty minutes, dilution gives an olive to dark green color, and organic solvents extract the same colors as when the heating has been carried to  $150^\circ\text{C}$ , but weaker. If, after heating to a little over  $100^\circ\text{C}$ , ammonia is added to the diluted solution it becomes salmon pink, and amyl alcohol then extracts a pink color, leaving the aqueous layer light orange.

Heroin, codeine, and diionine give reactions in this test so much like that of morphine that they are not distinguishable. Unaccountably, codeine seems to give the test more readily than morphine.

While the perchlorate test is less complex, less distinctive, and less interesting than the arsenate test, it has one very considerable advantage over the latter: the reaction is not noticeably affected by the presence of chloride. The hydrochloride of the alkaloid, therefore, may be used as well as the sulphate or the free alkaloid. With bromide present, however, the "Halide-Oxidation Test" is obtained.

The perchlorate test should prove as useful as any, when one of this type is wanted.

## IV THE HALIDE OXIDATION TEST

Several references have already been made to the fact that the presence of chloride or bromide greatly changes the 'Arsenate Test' or the 'Chromate Test'. The purposive addition of sodium chloride, before oxidation by arsenate or chromate, provides an excellent test for morphine. Sodium bromide does even better, and can be used without the addition of an oxidizing agent, as hot sulphuric acid itself frees the halogen, in this case. Chloride, either with arsenate or chromate, and bromide with or without the addition of arsenate or chromate give results in the various combinations so similar that we certainly have to deal with only a single test, or reaction. For want of a better name, and to avoid any assumption in naming the test, as to the nature of the reaction, I have called it the Halide Oxidation Test.

**Bromide**—Put a little morphine or its salt in a clean dry test tube and add a little NaBr. Add 8 or 10 drops concentrated  $H_2SO_4$  and shake the tube slightly to ensure contact of the acid with the morphine. Stand the tube in the boiling water bath for about a minute. The colors obtained with solvents are practically the same regardless of the temperature used so this is the simplest method of heating. The color of the concentrated acid solution varies from dark green due to the morphine to red brown, due to the bromide. When taking the tube out of the water bath so that no morphine is floating undissolved on the acid. Dilution gives dark brown soon dark green. Dilution to the extent of about one half cc of water for each drop of concentrated  $H_2SO_4$  gives a solution with a strong bluish green color, in which a dark green precipitate soon forms. Amyl alcohol extracts deep green, benzol deep blue (or indigo). Chloroform on shaking well is colored at least green, with more morphine blue green to deep blue with much free bromine in the solution at first yellowish. Ether shaken with the solution which is still warm from the dilution, readily extracts a violet or violet blue color but as the solvent cools (which, of course, occurs quickly) it deposits a green sediment on the sides of the test tube losing most of its color and thus appearing to change to a weak gray. These colors are but little affected by addition of  $NH_4Cl$ ,  $NaHCO_3$ ,  $Na_2CO_3$  or  $NH_4OH$ .

The addition of an oxidizing solution of arsenate or chromate does not change the reaction, but assists in freeing bromine to which the test seems to be due. By this means the test can be carried out very easily at room temperature but if the solution is heated as described, the addition of an oxidizing agent is unnecessary.

**Chloride and Chromate**—To a little dry morphine or its salt in a test tube add a pinch of NaCl then 10 or 12 drops of concentrated  $H_2SO_4$ , shake the tube lightly then at once add 5 or 6 drops of an  $H_2SO_4$  solution containing 1 gm  $K_2Cr_2O_7$  per 100 cc. The concentrated acid solution is first dark brown, after warming (as described for bromide) dark greenish brown. Diluted slightly with water the color is red with further dilution the solution is clear light greenish yellow to light yellow green. Amyl alcohol extracts bright (bluish) green, chloroform greenish blue ether good violet and benzol lavender or violet.

Note that much more chromate is used than can be used in the chromate test. The chloride takes care of the excess. Of course the chromate must not be in excess of both the morphine and the chloride together. Furthermore it should not be added warm on very hot days the acids should be cooled beforehand. Chloride and chromate give the least satisfactory form of the test.

**Chloride and Arsenate**—Proceed as for chloride and chromate but for the solution of the latter substitute 4 or 5 drops  $H_2SO_4$  containing 5 gm  $KH_2AsO_4$  per 100 cc. A red brown color develops on warming dark brown or green brown. Dilution with a little water gives deep red further dilution oliveaceous green. From this latter solution amyl alcohol extracts a deep blue or blue green color often appearing blue on looking directly through the solution, but blue green when shaken up on the sides of the test tube. A yellow color is left in the aqueous layer. Chloroform extracts deep blue benzol indigo or violet, ether reddish violet. Reducing the acidity of the aqueous solution, even to making it slightly

alkaline, changes these colors very little, but greatly reduces the amount of color extracted by benzol or ether. Addition of  $\text{NH}_4\text{Ac}$  to the aqueous solution makes it green. Amyl alcohol then extracts deep green, chloroform good greenish blue.  $\text{Na}_2\text{CO}_3$  or  $\text{NaHCO}_3$  in excess makes the aqueous solution brownish pink to pinkish brown. Amyl alcohol then extracts a deep blue color, leaving the aqueous layer slightly pink.

#### V MISCELLANEOUS REACTIONS

There still remain undescribed in these articles some five tests or alleged tests for morphine or codeine which include heating in concentrated sulphuric acid solution. At the present time I am not considering any of the numerous tests which are carried out with cold concentrated sulphuric acid reagents. Froehde's reagent, Marquis' reagent, etc. The tests remaining are the phosphate, benzidine, arsenite, ferric chloride, and persulphate tests. They will now be described and discussed very briefly.

*"Phosphate Test"*—"If sodium phosphate is substituted for arsenate and heat applied until acid fumes appear, the mixture becomes violet changing to brown or olive-green. If after cooling, water be gradually added, a reddish-brown coloration appears, changing to dirty bluish-green on further dilution. On shaking with chloroform the latter is colored blue."—Allen's Commercial Organic Analysis. This supposed test is also given by the U S Dispensatory, which credits it to Vulpus.

So far as I have been able to discover, the addition of phosphate ( $\text{NaH}_2\text{PO}_4$ ) does not affect the sulphuric acid color test in the least, nor does it introduce any new effects.

It might be mentioned, however, that if the heating is carried out in an evaporating dish, instead of in a test tube, slightly different effects may be obtained, doubtless because in the evaporating dish the solution is more exposed to the air, which is capable of partially oxidizing the morphine-derivative in the hot sulphuric acid solution.

*"Benzidine Test"*—"On heating 0.01 to 0.02 gm morphine hydrochloride in a few c.c concentrated  $\text{H}_2\text{SO}_4$  containing 0.01 gm benzidine, the liquid is first yellow, changing to brown, greenish-brown, and finally dark green. On adding this solution drop by drop to  $\text{H}_2\text{O}$ , a bluish-violet color is obtained. On shaking with  $\text{CHCl}_3$ , the latter extracts bluish, with the aqueous layer rose-red.  $\text{NH}_4\text{OH}$  added to the violet solution yields blue changing to green then greenish-gray. Codeine, diionine, heroin, apomorphine are similar," etc.—Chem Abst, 1926, xx, 3330, credited to L Ekkert. Pharm Zentralhalle, 1926, lxxvii, 498.

I have not been able to obtain any effect with benzidine. Very likely there has been a mistranslation, but in any case the description is suspiciously like that for the sulphuric acid color test.

*"Arsenite Test"*—"Reichard states that if morphine is warmed gently with concentrated sulphuric acid containing arsenous or arsenic acid, an intense and permanent purple color is produced. The reaction is best obtained by dissolving the arsenous acid in a little strong solution of sodium hydroxide,

then add the morphine and finally an excess of concentrated acid"—Allen's Commercial Organic Analysis Blyth (Poisons, Their Effects and Detection) also gives this test

It is certain that no intense and permanent purple color is obtained as stated

Arsenous acid,  $As_2O_3$ , sometimes yields a pink or red color in such a test, more often, with morphine, no color at all (At one time I thought this a test for codeine) I have not yet discovered just what are the necessary conditions for producing a pink or red color in this test

Arsenic acid,  $As_2O_5$ , of course gives the arsenate test, but is not very convenient to use, as it does not dissolve readily in the sulphuric acid

Confusion of  $As_2O_3$  with  $As_2O_5$  is not uncommon An example is in Autenrieth Warren (Detection of Poisons) where Gugliemelli's Reagents are given with arsenic trioxide,  $As_2O_3$  I have tried naturally without success, to obtain the reaction claimed for apomorphine with Arsenotungstic solution, when the reagent is made as directed The real test is simply the oxidation test for apomorphine, combined with a deep blue color in the aqueous layer due to the reduction of the Arsenotungstic solution, made with  $As_2O_5$  The same remarks doubtless apply to the  $As_2O_3$  of the Arsenotungstomolybdic solution, though I have not tried it The mistakes possibly arise through mistranslation

*Ferric chloride test for Codeine*—Warmed with sulphuric acid containing a little ferric chloride, codeine gives a deep blue color This is one of the tests for identity of codeine in the U S Pharmacopoeia With only a little alkaloid heating to as much as  $80^\circ C$  may be necessary The color will develop only very slowly in the cold A convenient way of obtaining the reaction in ordinary cases is to dilute a drop or two of  $FeCl_3$  solution with  $H_2SO_4$  to a light yellow color and apply this reagent while still warm (from the dilution) to the codeine on a spot plate The blue color then develops at once if a fair amount of codeine is used Morphine in such a test gives no color Heated to  $80^\circ C$  in  $H_2SO_4$  containing  $FeCl_3$  morphine gives a comparatively weak, dirty violet color, or may even, under proper conditions give blue

Blyth says, "This blue color is apparently common to all ethers of the codeine class" (Poisons Their Effects and Detection) Even so the test is a fairly good one for distinguishing codeine from morphine

*Persulphate test*—If morphine is dissolved in concentrated sulphuric acid and a little ammonium persulphate in sulphuric acid added a yellow color is produced which changes to green Heating produces an orange brown color Dilution of the green (or orange brown) solution gives a yellow solution from which amyl alcohol extracts merely a yellow or orangish yellow color The reaction therefore has no qualitative interest aside from the green color developed in the concentrated acid Codeine reacts like morphine

## VI NATURE OF THE REACTIONS

*Apomorphine and Dehydrated Morphine*—When morphine is heated in concentrated phosphoric acid, in a saturated solution of zinc chloride, or in concentrated hydrochloric acid under pressure (or in the presence of a drop or two of concentrated  $H_2SO_4$ ), it is dehydrated, and apomorphine is formed When apomorphine is oxidized it is converted into a compound which is bright green in aqueous (nearly neutral) solution, crimson to magenta pink in ether, blue in amyl alcohol, etc This is known as Pellagri's test for morphine



When morphine is heated in pure concentrated sulphuric acid and the solution then cooled and treated with a tiny crystal of potassium nitrate or some similar oxidizing agent, a magnificent deep red color is obtained. This is known as Husemann's test. Most authorities intimate, and some of them say outright, that this reaction is due to apomorphine. It seems natural that dehydration by sulphuric acid should give the same compound as dehydration by phosphoric or hydrochloric acid. Nevertheless, this idea is entirely erroneous.

When morphine is dissolved in pure concentrated sulphuric acid and the solution warmed slightly, then diluted with water, oxidized with iodic acid, and neutralized, a bright green color is obtained, and amyl alcohol extracts blue. Thus far it seems as though apomorphine must have been formed, but ether, and also chloroform, benzol, etc., entirely fail to extract any color whatever. From the different solubilities of the oxidation product it is clear that the compound formed is not apomorphine.

A study of this "Derivative Oxidation Test," as I have called it, shows that the change begins as soon as the morphine is dissolved in the acid, and takes place gradually at room temperature, and that the green color in aqueous solution is strengthened (when only a few minutes are allowed for the action of the acid) if the concentrated acid solution is heated at least to  $35^{\circ}\text{C}$ , and reaches a maximum at not over  $50^{\circ}\text{C}$ .

When morphine is heated in pure concentrated sulphuric acid to  $40^{\circ}$  to  $50^{\circ}\text{C}$ , and the diluted solution tested with such general alkaloidal reagents as phosphomolybdic acid, Wagner's reagent, Mayer's reagent, and gold chloride, no precipitate is obtained, though these reagents are all very sensitive to morphine and to apomorphine also. This shows again that the compound formed is not apomorphine, and it also shows that morphine is completely converted to an entirely different compound by the action of sulphuric acid, in a few minutes and with very little heating. It would seem that the compound formed is not even an alkaloid, but there are several recognized alkaloids which are not precipitated by most of the general alkaloidal reagents. When the diluted acid solution is allowed to stand, without oxidation or other treatment, the morphine derivative is precipitated, a colorless, crystalline compound. Its color reactions are similar to, some identical with, those of apomorphine, including a color reaction with ferric chloride. Like morphine and apomorphine, but unlike most alkaloids, it is readily soluble in bases.

The statements in the textbooks as to the compound formed on heating morphine in sulphuric acid seem to be nothing more than poor guesses. As already mentioned, several authorities say that apomorphine is formed, on heating at  $100^{\circ}\text{C}$ . Witthaus (Manual of Chemistry) says that di- and trimorphine are formed at that temperature. Prescott (Organic Analysis) says that on heating at  $150^{\circ}$  to  $160^{\circ}\text{C}$  sulphmorphide is formed, a substance similar to apomorphine sulphate. As we have seen, a far less degree of heat than is usually given is sufficient to effect a complete change, and a change more radical than any of those indicated. It may be a change of polymerization or molecular rearrangement, or possibly even one of mild oxidation, but

since the color reactions of the compound formed are so similar to those of apomorphine it seems most probable that this compound also is dehydrated morphine. Since it is not identical with apomorphine, we must suppose that molecular rearrangement or polymerization takes place in addition to dehydration. One authority (I cannot locate the passage at the present time) suggests that apomorphine itself is polymerized. No doubt these problems can be solved easily by analysis, but thus far I have had time only for working out the qualitative tests.

*Dehydrated Codeine and Apocodeine*—When codeine is warmed in pure concentrated sulphuric acid to  $40^{\circ}$  to  $50^{\circ}$  C, and the solution cooled and diluted a crystalline compound is precipitated on standing just as with morphine. Codeine gives the derivative oxidation test the same as morphine. Now codeine itself differs from morphine qualitatively in two respects: it is not a reducing agent (in aqueous solution) and so does not respond, for instance, to the test with iodic acid and ammonia and it has no phenolic hydroxyl group, and so gives no color with ferric chloride. The codeine derivative formed in sulphuric acid at  $40^{\circ}$  C is a reducing agent, being readily oxidized, for instance, by iodic acid. But it is not a phenol if we may judge by its failure to react with ferric chloride whereas the morphine derivative shows the same phenolic color reactions as apomorphine.

Thus dehydrated codeine is quite distinct from dehydrated morphine, at least when the dehydration is accomplished by means of concentrated sulphuric acid. The existence of apocodeine as a definite compound has been called in question. "D. B. Dott doubts the existence of apocodeine, and states that commercial *apocodeine hydrochloride* is not of a very definite nature, being probably a mixture of an amorphous modification of codeine, polymerized bases, chlorocodide and apomorphine"—Allen's Commercial Organic Analysis. It is probably significant that Stephenson in his investigation of microchemical tests for all aloids was unable to obtain a single crystal with apocodeine, although it gave precipitates with all the common alkaloidal reagents. Certainly this is not suggestive of a pure substance.

All this may be true of commercial apocodeine, but since codeine retains one of the two characteristics by which it differs from morphine when dehydrated by sulphuric acid, it seems to me that it should retain the same characteristic difference when carefully dehydrated by phosphoric acid. Experiment bears this out, for when codeine is boiled just a moment in 85 per cent phosphoric acid it will give the oxidation test (Pellagris), but not the iron test, whereas with morphine the two tests go together. To be sure, some authorities say that when dehydrated by concentrated hydrochloric acid codeine first forms chlorocodide, and then apomorphine. The whole subject is presented in a very confused and contradictory manner, I may at least succeed in getting some one who really knows to clear it up.

In *Chemical Abstracts* for 1926 (v, 1469), I notice the following: "Apparently the experiments hitherto performed with apocodeine have been made with impure preparations. Crystalline apocodeine is some five times as active as the amorphous commercial apocodeine. On the other hand there is no im-

portant qualitative difference between the (pharmacologic) action of the pure and the commercial preparations, etc"—Otto Kraye, Arch Exper Path Pharmacol, 1926, cxi, 60-7

*Changes on Further Heating, and in the Presence of Oxidizing Agents*—In Husemann's test the directions are, usually, that the morphine be dissolved in sulphuric acid and heated to, or at, 100° C. If heated only to 40° the reaction is not quite the same. In the "Derivative Oxidation Test" the morphine is heated in sulphuric acid to 40° C. If heated to 100° instead, the same green color is produced in the aqueous layer when the test is applied, but amyl alcohol will no longer extract any blue color whatever. A study of these and other reactions shows that when morphine (or codeine) is heated in pure concentrated sulphuric acid a change takes place at 60° to 70° C. It is a minor change, a slight rearrangement of the atoms in the molecule, or a change in polymerization. The derivative will no longer crystallize out of the diluted solution.

On continuing the heating to 140° and above, a dark green color develops. As dehydration has already taken place, presumably, we naturally think of decomposition or oxidation, or both. Hot concentrated sulphuric acid is an oxidizing agent, and at the temperature of the change it is very nearly fuming. Morphine itself is so readily oxidized as to be classed as a reducing agent, when dehydrated it is still more easily oxidized, it is, therefore, not surprising that hot sulphuric acid oxidizes it at 140°.

The sulphuric acid color test reaches its maximum about 175° C, with a slight change in the colors obtainable in organic solvents occurring at about 165°. Now when arsenate, chromate, or perchlorate is present, we have oxidation taking place even below 100°, but this does not prevent the formation of practically the same colors above 140°, as in the sulphuric acid color test. It seems, then, that on heating in pure sulphuric acid, oxidation takes place at 140° in much the same way as in the presence of chromate at the same temperature, or with perchlorate at 125°, or with arsenate at as low as 115°. The change at 165°, which takes place in all four cases, is then probably one of decomposition. It takes place subsequently to the oxidation when an oxidizing agent is present, but concurrently with the oxidation which has begun but which at 165° is still incomplete, in pure sulphuric acid.

Chromate is an energetic oxidizing agent, and can be present only in very small amount. Concurrent oxidation and dehydration are then produced. Arsenate is a milder but very effective oxidizing agent. That the action of the two is substantially the same is shown by the similar effects produced in the chromate test and the arsenate test, and further by the fact that in the presence of chloride or bromide the same reaction is obtained with both. Perchlorate is an oxidizing agent still milder (at low temperatures) than arsenate, with its dehydration taking place before oxidation, but in the same solution and in the same continuous operation. In the presence of arsenate or chromate the same kind of change takes place at 60° to 70° C that we have already noted for pure sulphuric acid, and these temperatures also mark the beginning of the perchlorate test.

The weak blue color that is first obtained in the arsenate test, likewise the dark brown or red brown first produced by chromate, seems to be due to the oxidation of the morphine as such. It does not interfere with the subsequent dehydration.

Sodium chloride when warmed with chromate in sulphuric acid is of course oxidized, chlorine being set free. Apparently it is the free chlorine or bromine which makes the halide oxidation test distinctive and different from the arsenate or chromate test. No new effect could be found with sulphate added, in the chromate test. Thus in the presence of chloride, the oxidizing agent actually added operates only indirectly, and an indirect

THE CHANGES WITH TEMPERATURE ON HEATING MORPHINE IN CONCENTRATED SULPHURIC ACID\*

	HEATING IN PURE $H_2SO_4$	ARSENATE TESTS	CHROMATE TEST PERCHLORATE TEST
15 - 20 C	Morphine gradually converted to comp insol in dil acid, and with color reactions like Apomorphine	Morphine gives blue color in $H_2SO_4$ -Ars sol	Chromate test Amyl alc ext green from dil sol other solvents no color
30 - 40 C	Same change more quickly	Morphine color becomes green in $H_2SO_4$ -Ars sol	Chromate test same reaction sooner complete
50 C		Dionine gives purple color	
60 - 70 C	Compound becomes sol in dil acid minor changes in color reactions occur	Morphine color becomes blue in $H_2SO_4$ -Ars sol	Chromate test $CHCl_3$ ether, benzol hereafter ext colors from dil sol Perchlorate test begins, deep violet in conc acid sol develops
75 - 80 C	Dionine derivative begins to react like that of morphine to $FeCl_3$	Codeine gives purple color Dionine begins to react like morphine	
100	Codeine derivative begins to react like that of morphine to $FeCl_3$	Morphine gives green on diln and org solvents ext colors. Codeine begins to react like morphine	
105			Perchlorate test green on diln and org solv ext colors
115		Color changes to green in $H_2SO_4$ -Ars sol, Organic solv ext strong colors	
125			Perchlorate test color changes to green in conc acid sol, Amyl alc ext deep color and other solvents some
140	$H_2SO_4$ sol becomes dark olive green Amyl alc ext slight violet from dil sol		Chromate test colors on diln and extn with Amyl alc practically as with heating in pure $H_2SO_4$ stronger
160	Amyl alc ext blue from dil sol green from ammoniacal sol	Colors extracted by organic solvents change in amylnic nearly same as with heating in pure $H_2SO_4$	Chromate test colors change as with heating in pure $H_2SO_4$ nearly Perchlorate test colors change as with heating in pure $H_2SO_4$ nearly
180	Charring set in		

\*Note Temperatures are approximate and show when changes have noticeably begun not when the effects are strongest

oxidation can be effected by concentrated sulphuric acid even at low temperatures, through the medium of sodium bromide

It is just when one would least expect it, in sulphuric acid solution, that it makes a great difference whether the morphine is in the form of the hydrochloride or the sulphate

#### SUMMARY

**I Sulphuric Acid Color Test** Morphine gives a color reaction on heating in pure concentrated sulphuric acid to  $140^{\circ}\text{C}$  and above. For a qualitative test the heating is carried to  $175^{\circ}$ . The colors extracted by amyl alcohol from the diluted solution are especially useful. Codeine gives the same test.

**II Arsenate Test** Morphine gives three qualitative color tests with sulphuric acid containing a little arsenate, as follows

- 1 A succession of colors in the concentrated acid solution on heating
- 2 Distinctive colors extracted from the diluted solution by organic solvents, after heating to  $130^{\circ}\text{C}$
- 3 Distinctive colors extracted from diluted solution by organic solvents, after heating to  $175^{\circ}\text{C}$

The second is the best qualitative test, and an excellent one. Codeine does not give quite the same reaction as morphine. Although many authorities mention the arsenate test, existing descriptions in books are without statements of the requisite temperatures, and fail to recognize the interference of chloride or bromide.

**III Chromate Test and Perchlorate Test** Morphine gives a reaction with chromate similar to that with arsenate. Four phases are distinguished, providing separate tests. Colors extracted by solvents after heating to (1)  $40^{\circ}$ , (2)  $100^{\circ}$ , (3)  $155^{\circ}$ , (4)  $175^{\circ}\text{C}$ . The chromate must not be in excess of the morphine, and the test is not of much practical value.

Perchlorate gives a test similar to the arsenate test, but not so complex. No color develops until the concentrated acid solution has been heated to about  $60^{\circ}\text{C}$ . Two qualitative tests are distinguished, as follows

- 1 Distinctive colors obtained by heating to about  $150^{\circ}\text{C}$
- 2 Distinctive colors obtained by heating to nearly  $175^{\circ}\text{C}$

The first of these tests is the better, and should be a useful test of this type. Chloride does not interfere with the perchlorate test.

**IV Halide-Oxidation Test** Morphine when heated with sodium bromide in concentrated sulphuric acid solution gives a colored product which organic solvents extract from the diluted solution with bright distinctive colors. Codeine gives the same test. This reaction appears to be due to the bromine set free by the concentrated sulphuric acid, for when chloride is substituted for bromide, and an oxidizing agent (arsenate or chromate) added, practically the same reaction is obtained. This is a new and sensitive qualitative test. Because of this reaction, it is desirable and to some extent necessary

that morphine (or codeine, heroin, diuine) for the arsenate (or chromate) test should not be in the form of the hydrochloride

V Miscellaneous Reactions A brief discussion is given in this article of the reactions or supposed reactions of morphine and codeine on heating with phosphate, benzidine, arsenite, ferric chloride, or persulphate, in sulphuric acid solution

VI Nature of the Reactions In this article the relationship of the various tests is discussed together with the changes produced on heating morphine in pure sulphuric acid An attempt is made to explain the changes taking place in these tests as the result of dehydration (although no apomorphine is formed), due to the concentrated sulphuric acid, with concurrent oxidation (in the reactions discussed in the present paper), due to the arsenate, chromate or other oxidizing agent added

General As qualitative tests, the following are the best of those described

- 1 The Halde Oxidation Test using bromide
- 2 The Perchlorate Test, with heating to about 150° C
- 3 The Arsenite Test when the alkaloid is present as the sulphate or is free, with heating to about 135° C

The interference of chloride should be considered a possibility, prior to investigation, in all tests in which morphine or one of its derivatives is oxidized in concentrated sulphuric acid solution As heroin and diuine are usually, and morphine often sold in the form of the hydrochloride, this is an important matter

It has become evident that each oxidizing agent has its characteristic reaction with morphine Additional tests similar to those described in this paper will no doubt be discovered in the course of time, but it is hardly worth while to look for them Plenty of good tests for morphine are now known, it is only necessary to separate the good from the bad An authoritative general review of the tests for morphine should be made, and then analytic investigators should turn their attention to other alkaloids

#### REFERENCES

- <sup>1</sup>Allen's Commercial Organic Analysis ed 4, 1912
- <sup>2</sup>Blyth Poisons, Their Effects and Detection, ed 5 1920
- <sup>3</sup>Autenrieth Warren Detection of Poisons, ed 5, 1921
- <sup>4</sup>Fuller Chemistry and Analysis of Drugs and Medicines, 1920
- <sup>5</sup>United States Dispensatory, ed 20, 1918
- <sup>6</sup>United States Pharmacopoeia X, effective 1926
- <sup>7</sup>Fresenius Wells Qualitative Chemical Analysis, ed 16, 1915
- <sup>8</sup>Prescott Organic Analysis, 1887
- <sup>9</sup>Perkin Qualitative Chemical Analysis, 1901
- <sup>10</sup>Peterson, Haines, and Webster Legal Medicine and Toxicology, II ed 2, 1923
- <sup>11</sup>Witthaus Manual of Chemistry, 1902
- <sup>12</sup>Stephenson Some Microchemical Tests for Alkaloids, 1921
- <sup>13</sup>Levine and Magiera Studies in Toxicologic Chemistry, II The Formaldehyde Sulphuric acid Reaction of the Opium Alkaloids JOUR LAB AND CLIN MED, May 1927 xii 773
- <sup>14</sup>Fulton Some New and Improved Tests for Morphine and Related Alkaloids JOUR LAB AND CLIN MED May, 1928, xiii, 750

## DISEASES OF THE MOUTH\*

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**H**EALTH is our greatest asset. A clean mouth and sound teeth have a tremendously important bearing on the physical condition and efficiency of an individual. The mouth is subject to as great a variety of diseases as any other part of the system, although their multiplicity is not always appreciated. Diseases of the mouth may be either local in character or they may constitute part of the picture of general disease. While it has been recognized by many for years that oral sepsis causes many systemic disturbances, it has been only recently that we have learned to know the advantages and real necessity for a close cooperation between physicians and dentists in handling these cases. The dentist has learned that he may usually expect the most unselfish cooperation from the physician, and in return it is expected of him that he limit his activities where the service can be better performed by the physician, surgeon, or specialist, and endeavor to aid the medical man in his effort to eliminate all pathologic mouth conditions.

While it has been common practice in the past for the dentist to consider the maxillary sinus his special field, I am sure it has been our experience in Washington and in many other localities, that the most good may be accomplished by the dentist handling the mouth factors and lending any further service he can to the rhinologist. This is true of many other medical and surgical problems. I believe that the sooner mouth wounds communicating with the maxillary sinuses are closed, and further treatment turned over to the rhinologist, the better the results.

In the study of treatment of diseases of the mouth, I believe it is generally conceded that diagnosis is of paramount importance. A correct diagnosis is imperative before the prognosis can be given and the proper treatment instituted. There can be but one true diagnosis, but many forms of treatment in the hands of different men may accomplish the same result. A thorough understanding of all of the diagnostic methods is essential for the successful treatment of a patient who has come to the physician or to the dentist for the relief of disturbing symptoms.

A mouth examination should be conducted in a routine manner and the results recorded upon some suitable record chart.

A very common misconception of the proper diagnostic methods is the practice of many physicians in referring patients to the dentist for a radiographic survey of the mouth with a request for a radiographic report, and there is a very prevalent idea that the only prerequisite for a mouth diagnosis is the ability to take good radiograms. If a radiographic examination is to

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be of any great value it should be taken by a dentist or physician schooled in x-ray technology and the fundamental principles of diagnosis, and whose experience in this field gives a more correct interpretation. The fact should not be overlooked that radiograms of the teeth are in most cases useless without considering the findings of the other diagnostic methods such as history, manifestations of pain, general oral examination percussion and palpation, exploration, color, conductivity of temperature, transillumination, electric pulp test bacteriologic examination, histopathologic examination, blood, and other laboratory methods. There also seems to be a very general idea that the pulpless tooth is the only source of oral sepsis, causing systemic disturbances. This is only one of many factors. A vital tooth may in some instances show periapical disease. Periodontal diseases, and especially periodontoclasia, are present in the mouths of most adults.

Other etiologic factors to be considered are stomatitis of various types, impacted and unerupted teeth fractures, periostitis rarefying and condensing osteitis, acute and chronic osteomyelitis necrosis osteitis serrata exostosis, myositis ossificans radicular follicular and multilocular cysts and benign and malignant tumors, etc.

The value of radiography should not be misunderstood. It is relative, as one views the abnormal area by contrast. A radiolucent or radiopaque area may be hidden or distorted by exposure from different angles interposing tooth roots or dense bone and thus many infected areas may be overlooked. Pathologic areas and areas undergoing regeneration following operation should be compared with other similar areas in the mouth and contrasted. Good radiograms are rarely misleading if properly interpreted but their possibilities are limited and the vital factor in determining the presence or absence of disease must be the final clinical examination. It has been my experience that the postoperative results are usually more pronounced than the radiogram would indicate.

Inflammation is a cellular defensive reaction against injury, and infection is not the only element causing this injury as it may be due to trauma chemicals, heat, cold, or electricity.

As a result of inflammation rarefaction or condensation of bone may result, or they may both occur simultaneously. Therefore rarefaction is indicative of inflammation, which may possibly be from infection and causes the radiolucent radiographic appearance. Inflammation may cause an increased density of the bone lamella and results in a radiographic radiopacity.

The electric pulp tester is a valuable diagnostic agent when used with transillumination and the radiogram but is not reliable when used independently.

Transillumination of the teeth gums, and sinuses is indispensable. The radiogram may be absolutely negative as there may be no bone pathology shown and transillumination may show definite disease and in some cases both of these methods may be negative and disease may be present. Transillumination is a valuable aid in making a diagnosis of mouth conditions, but it has its limitations. It is unreliable in disclosing areas of periapical infection, and it does not disclose bone pathology, except where it extends to the



soft tissues In other words, the pathology, shown by transillumination, is due to soft tissue infection

The fact that the alveolus and gums do not show a shadow, when transilluminated, does not eliminate the possibility of infection My experience is that it is usually not possible to detect periapical infection by transillumination where the periapical disease is not accompanied by periodontal disturbances

One of the perplexing problems of oral diagnosis is the unerupted or impacted tooth, as it should always be considered questionable even though no definite symptom of pathology is shown While it is possible in some instances for pressure of an impaction to give reflex disturbances, I do not believe this usually occurs without local symptoms, but I do believe that the osteitis and infection about the impaction are the important etiologic factors so far as systemic disturbances are concerned

The removal of impacted third molars more than any dental operation is attended with serious consequences Anyone who has attempted much of this work will have experienced some very serious results While, as many of you realize, this in many cases is a dangerous procedure, it is difficult to educate the public that it is as important to have the proper postoperative care in these cases as in tonsil and other operations

It takes experience to teach one that it is better not to remove one of these teeth, than to do so and have the patient not remain or fail to return for treatment I believe we will learn to hospitalize these patients more and more

A periodontal disease is a disturbance of the periodontal tissues It includes all diseases of the tissues surrounding the tooth root which have their origin in the gingivae, periodontal membrane, and gums

The term "pyorrhea" which is quite widely used in speaking of these conditions has given rise to a great deal of confusion, as pyorrhea, or Rigg's disease, is described as a loosening of the teeth associated with continued suppuration There are various stages of periodontal involvement, and the initial lesion is not associated with suppuration, but only with simple inflammation The term "pyorrhea," therefore, does not convey any idea as to the state of involvement of the tissues Under periodontal disease we consider the different forms of recession, gingivitis, hypertrophy, pericementitis, and periodontoclasia

Unfortunately the pulpless tooth problem is one of the phases of our work upon which there is a difference of opinion

Radiographically the pulpless tooth may be functional and harmless if the lamina dura is definite, if the surrounding bone cancellations appear to be normal, as compared with adjacent normal teeth, if there are no clinical symptoms of disturbance, if the apex is not hypoplastic and there is a filling well into, but not through, the apex

In doubtful cases where the vitality test, transillumination, radiograms, and other methods do not show definite infection and it is suspected, the health of the individual must be taken into consideration The pulpless tooth is in the same category as the tonsils The diagnosis cannot always

be made from the local appearance, but general symptoms must be considered. It is not always possible to examine a tonsil or a pulpless tooth and give a positive assurance that there is no pathology, even in the absence of any apparent infection. Where sepsis is known to exist, and other foci have been eliminated or ruled out, it may be necessary to remove pulpless teeth or to open into suspected pathologic bone areas throughout the mouth, even though no definite infection is apparent.

I do not agree with the radical who condemns every pulpless tooth. I am not pleading for the pulpless tooth, for I am very much afraid of it, but I do believe if the patient will present himself in time, and the pulp is removed aseptically and the canals filled properly that a large percentage of such teeth may be retained. I do not believe an infected tooth apex should be retained a minute, and I would consider it a waste of time to attempt to treat one unless an apicectomy is performed in a selected case.

In determining when to extract a pulpless tooth it is necessary to take into consideration many factors. Each case should be handled as good judgment and experience indicate, and with but very few exceptions, my practice is to recommend extraction of

- 1 All teeth showing evidence of periapical bone involvement
- 2 All pulpless teeth in patients whose health is seriously endangered
- 3 All pulpless teeth showing marked periodontal bone resorption (periodontoclasia)
- 4 All pulpless teeth directly bordering on the maxillary sinus
- 5 All third molars when pulpless or causing pressure or decay in other teeth
- 6 All pulpless teeth when the root formation precludes the possibility of a good root filling
- 7 All pulpless deciduous teeth

If I were to make any specific recommendation for proper cooperation of physician and dentist, I would call attention to the advisability of personal contact. The patient cannot be trusted with reports, as they are so apt to misinterpret them. If the physician refers a case to the dentist, it would be well to telephone or write to him giving him some idea of the patient's general condition, and what is to be determined. In many cases, it is necessary for the dentist to see the patient after radiograms and other evidence are completed so that a proper diagnosis may be made and an intelligent report and recommendation made. Patients should be taught the value of preoperative and postoperative radiographic examination, as well as laboratory and other diagnostic methods.

It is not enough that a physician advise a patient to go to his dentist and have his teeth looked over. In many instances the patient is at fault when the physician receives an unsatisfactory report, as he fails to advise the dentist what is wanted.

It is inconceivable and unfortunate that after more than fifteen years of close study on the part of medicine and dentistry, there is still a divided

opinion regarding the rôle of oral sepsis. Dentistry has many unsolved problems and much should be accomplished in the field of prevention, and care of the mouth.

We hear a great deal about education of the public in regard to early recognition of stomatitis and tumors of the mouth, but I wonder just how many physicians and dentists are able to diagnose these mouth conditions when they see them. I believe that it is apparent to all that there is great need for a more thorough study of mouth conditions, and proper training of both the physician and the dentist, to enable him to recognize the character and significance of the many oral abnormalities.

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## PARATHYROID EXTRACT AND GLUCOSE TOLERANCE IN DIABETES MELLITUS\*

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IN A PREVIOUS paper one of us<sup>1</sup> discussed the influence of calcium on carbohydrate metabolism. It was shown that there occur simultaneously changes in calcium and carbohydrate metabolism in disturbances of the adrenal, thyroid, parathyroid, and pituitary glands and in various miscellaneous pathologic states. The latter half of the paper was devoted to the discussion of the relationship of calcium to carbohydrate metabolism in diabetes mellitus. It will be well to review some of the statements made.

Von Möræzewski<sup>2</sup> found an inordinate excretion of calcium in one case of diabetes mellitus. A year later he reported cases where the excretion of sugar was reduced by the daily ingestion of ten grams of calcium phosphate.<sup>3</sup> Von Noorden<sup>4</sup> observed uniformly a negative calcium balance in diabetes. This was out of proportion to an increased calcium elimination that might be caused by an existing acidosis. Falta and Whitney<sup>5</sup> found that pancreatectomy was associated with an immediate and rapid elimination of calcium. This was far in excess of that which could be accounted for by the mild acidosis present. The most elaborate work on the relationship between calcium and diabetes mellitus was performed by Kahn and Kahn.<sup>6</sup> They first showed that out of five cases examined all showed a definite negative calcium balance. The patients were kept on a standard diet for three days during which time the glycosuria and glycemia were determined. On the same diet they were then injected intravenously with varying amounts of eighth molecular calcium chloride every few days. The glycosuria and glycemia were determined to observe the effects of the treatment. As a result of these experiments they concluded that calcium administration definitely reduces glycemia and glycosuria in diabetes. More recently the following work has been done. Davies,

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Dickens, and Dodds<sup>7</sup> observed that in rabbits the injection of insulin produced a decided increase in blood calcium. Rothchild and Jacobsen<sup>8</sup> observed the same finding in man, this could not be confirmed by Harrop and Benedict.<sup>9</sup>

Employing the glucose tolerance test as an index of the severity of diabetes, it was shown by one of us<sup>1</sup> that calcium administered intravenously in no way ameliorates the diabetic state.

It was decided to continue this work and study the influence of parathyroid extract on glucose tolerance in diabetes mellitus. This subject is interesting from two standpoints: first the parathyroids are of the glands of internal secretion and many of the latter are intimately associated with carbohydrate metabolism, and secondly, the parathyroids exert a potent influence on calcium metabolism or to put it in the words of Collip<sup>10</sup> 'The function of the parathyroid hormone appears to be that of a regulator of calcium metabolism, and its action is primarily as a calcium mobilizer.'

Eppinger, Falta, and Rudinger,<sup>11</sup> also Marine,<sup>12</sup> observed a diminished tolerance for glucose in parathyroidectomized dogs but not in the tetany of man. Underhill and Blatherwick<sup>1</sup> observed that thyroparathyroidectomy causes hypoglycemia and that the normal glucose level is restored by calcium injections. They concluded that calcium may play an important role in maintaining the equilibrium of the blood sugar regulating mechanism.

In discussing carbohydrate metabolism two important phases must be considered: one, the oxidation of glucose which can be studied from the respiratory quotient, and two, the storage of carbohydrates (glycogenesis and glycogenolysis). These two phases are separate and distinct. Adrenalin engenders an increase in the oxidation of glucose and glycogenolysis. Insulin favors glycogenesis and an increase in the oxidation of glucose. Cammidge and Howard have made some interesting studies on the influence of the various hormones on carbohydrate metabolism. Only those findings that are relevant will be mentioned. It was found<sup>13</sup> that the injection of parathyroid extract into fasting rats and guinea pigs caused no change in the respiratory quotient. The injection of parathyroid extract and one half unit of insulin, however, produced a change in respiratory quotient and oxygen consumption resembling that previously found to follow the injection of two units of insulin alone. They studied also<sup>14</sup> the influence of the various hormones on the glycogenolytic activity of diastase *in vitro*. Adrenalin accelerates glycogenolysis. Thyroid extract has no influence on glycogenolysis, but it markedly augments the action of adrenalin. Insulin diminishes glycogenolysis. Parathyroid extract by itself has no influence on glycogenolysis, but it increases considerably the inhibiting action of insulin. They conclude as follows: "It would seem that the parathyroids are related to the internal secretion of the pancreas in much the same way as the thyroid is to the internal secretion of the adrenals."

Winter and Smith<sup>15</sup> observed that parathyroid extract alone had no effect on blood sugar concentration, but when combined with insulin only one third to one fourth the usual dose of the latter was necessary to induce convulsions. Forrest<sup>16</sup> also noticed that parathyroid extract had no influence on the blood

b This is also true of the forty-five minute postebum blood-sugar concentration

c In the two-hour postebum blood-sugar concentration one case shows no change, in four, that of the second curve is higher than that of the first, and in six, the reverse is true

d In the three-hour postebum blood-sugar concentration two cases show no change, in five, that of the second curve is higher than that of the first, and in four, the reverse is true

One is forced to conclude that parathyroid extract does not appreciably influence the blood-sugar curve

e Glycosuria.—Three cases show an increase in glycosuria after parathyroid administration, four a decrease, and two no change

#### SUMMARY AND CONCLUSIONS

The literature on the relationship of calcium to diabetes mellitus and of the parathyroids to diabetes and carbohydrate metabolism in general has been revived. One would gather from the work of Cammidge and Howard<sup>13 14</sup> and others that although by itself parathyroid extract has no influence on carbohydrate metabolism, it distinctly augments the potency of insulin. The administration of glucose causes a secretion of insulin into the circulation. The glucose tolerance test may be considered an index of the amount of insulin secreted. If parathyroid extract increases the potency of insulin, this should make itself evident in a reduction in the height and length of the blood sugar curve and in the amount of glycosuria. We did not find this to be the case. Our results are completely at variance with those of Cammidge and Howard and others. If we are to assume that parathyroid extract—Collip constitutes the sole active principle of the parathyroids, then we are forced to conclude that the latter are not concerned with carbohydrate metabolism.

We are indebted to Dr. Max Lederer, director of the laboratory, for helpful suggestions, and to Dr. Edmund Shlevin for the use of the cases in his clinic.

#### REFERENCES

- <sup>1</sup>Wishnofsky, M. Studies in Calcium and Carbohydrate Metabolism. I. Calcium and Glucose Tolerance in Diabetes Mellitus, *Jour. Lab. and Clin. Med.*, 1927, *xii*, 133-138.
- <sup>2</sup>Von Moraczewski, W. Stoffwechselversuch bei Diabetes Mellitus, *Centralbl. f. inn. Med.*, 1897, *xviii*, 921-932.
- <sup>3</sup>Von Moraczewski, W. Stoffwechselversuch bei Diabetes Mellitus, *Ztschr. f. klin. Med.*, 1898, *xxxiv*, 59-65.
- <sup>4</sup>Von Noorden, C. *Metab. and Pract. Med.*, 1907, *iii*, 600.
- <sup>5</sup>Falta, W., and Whitney, J. L. Zur Kenntnis des Eiweiss- und Mineralstoffwechsels pankreatischer Hunde, *Beitr. z. chem. Phys. u. Path.*, 1908, *i*, 224-228.
- <sup>6</sup>Kahn, M., and Kahn, M. H. Lame Deficiency of Diabetes Mellitus, *Arch. Int. Med.*, 1918, *xviii*, 212-227.
- <sup>7</sup>Davies, D. T., Dickens, F., and Dodds, E. C. Observations on Preparation, Properties, and Source of Parathyroid Hormone, *Biochem. Jour.*, 1921, *x*, 695-702.
- <sup>8</sup>Rothchild, F., and Jacobsohn, M. Der Einfluss von Insulin auf die Blutzusammensetzung beim Diabetes Mellitus, *Ztschr. f. klin. Med.*, 1926, *civ*, 70-79.
- <sup>9</sup>Harrop, G. A., and Benedict, E. M. The Participation of Inorganic Substances in Carbohydrate Metabolism, *Jour. Biol. Chem.*, 1924, *lix*, 683-697.
- <sup>10</sup>Collip, J. B. The Calcium Mobilizing Hormone of the Parathyroid Glands, *Jour. Am. Med. Assn.*, 1927, *lxxviii*, 565.

- <sup>11</sup>Eppinger, Falta, and Rudinger Quoted in Barker's Endocrinology and Metabolism, 1922, 1 637
- <sup>12</sup>Marine Quoted in Barker's Endocrinology and Metabolism, 1922, 1 635
- <sup>13</sup>Cambridge, P J and Howard, H A H Interrelation of Insulin and other Gland Extracts in Metabolism, Jour Metab Research 1924, vi 189
- <sup>14</sup>Cambridge, P J and Howard, H A H Influence of Insulin upon Activity of Diastatic Ferment of Liver Jour Metab Research 1925, v 95 105
- <sup>15</sup>Winter, L B, and Smith, W On a Possible Relation between the Pancreas and the Parathyroids, Jour Physiol 1925, lvi 108 110
- <sup>16</sup>Forrest, W D Effect of Parathyroid on Blood Sugar Curve After Insulin Brit Med Jour, 1923, ii 916 917
- <sup>17</sup>Petty, O H, Stoner W H and Schaffer, H W Clinical Application of Parathyroid Extract, 1926 xiii, 173 182
- <sup>18</sup>Kramer B, and Glittelman, I F Technique for Quantitative Estimation of Glucose in Small Amounts of Blood Jour Am Med Assn 1923 lxxxi, 1171
- <sup>19</sup>Foster, G L Some Comparisons of Blood Sugar Concentration in Venous Blood and in Finger Blood Jour Biol Chem 1923 lv, 291 301

544 WILLIAMS AVENUE

## NEW IODO DERIVATIVES OF PHTHALEINS\*

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**H**ALOGENATED phthaleins have recently aroused a great deal of interest. They have been introduced for cholecystography by E A Graham and his coworkers.<sup>1</sup> He investigated tetraiodophenolphthalein and tetrabromophenolphthalein. Today tetraiodophenolphthalein as a sodium salt tetrabromophenolphthalein, and tetrachlorophenolphthalein are prepared and marketed by a number of American manufacturers. They are being used in cholecystography.

The very unpleasant side reactions that occur after intravenous injection of these compounds show that they are still very toxic, and hence a search for new iodine compounds in this series of phthaleins was indicated. The iodine compounds were preferably synthesized on account of the high atomic weight of iodine and for the purpose of cholecystography was absolutely essential to make these drugs efficient.

Previous to this time, halogenated phthaleins have been considered important from two points of view first as indicators, and second for spectra absorption. Tetrabromophenolphthalein and tetraiodomofluorescein were studied by D L Pratt, F B Doam, and A W Harvey.<sup>2</sup>

For this investigation the two different isomers of tetraiodophenolphthalein, the  $\alpha$  tetraiodophenolphthalein and then the  $\beta$  tetraiodophenolphthalein were prepared. Then a completely saturated iodine compound of phenolphthalein was synthesized, namely, octaiodophenolphthalein, which as far as I am aware has not been prepared before. This compound should prove of immense interest in cholecystography as it has the highest molecular weight of them all. Its exceedingly high content of iodine should prove very advantageous in this field.

sugar level, but that when it was combined with insulin it caused a more marked lowering of the blood-sugar concentration than when insulin was given alone. Petty, Stone, and Schaffer<sup>17</sup> did not find it possible to reduce the insulin dosage in severe diabetics by using parathyroid extract.

It may be stated that the administration of glucose causes an outpouring of insulin into the circulation of all individuals except absolute diabetics. The amount of insulin discharged depends on whether the individual is normal or diabetic, and in the latter on the severity of the diabetes. If we are to accept the findings of Cammidge and Howard,<sup>13, 14</sup> namely, that parathyroid extract augments the capacity of insulin for glycogenesis and the oxidation of glucose, then the carbohydrate tolerance of a diabetic to whom glucose and parathyroid extract have been given should be increased. This paper is devoted to the study of the influence of parathyroid extract on the glucose tolerance test in diabetes mellitus.

A direct and accurate method of estimating the tolerance of an individual for carbohydrates is the glucose tolerance test. A normal individual when given 175 grams of glucose per kilogram of body weight on a fasting stomach shows a blood-sugar curve as follows: the maximum increase usually occurs at the end of one-half hour and is never more than from 30 to 50 per cent above the fasting level, at the end of two hours the fasting level should be regained. No glycosuria should occur. In diabetics the curve is equally characteristic, the maximum increase is much greater and the fasting level is not regained for many hours. Glycosuria occurs depending on the height and length of the curve and the renal threshold of that individual for glucose. The indices of the severity of any case therefore are the height and length of the blood-sugar curve and the amount of glycosuria.

The influence of parathyroid extract on the glucose tolerance test in diabetes was studied. Eleven cases suffering from diabetes of varying degrees of severity were chosen. The procedure was as follows: each patient after fasting fourteen hours received 100 grams of glucose in the form of a 50 per cent solution. Four blood specimens were taken: one, a fasting level immediately before ingestion and three at the following periods, forty-five minutes, two hours, and three hours after ingestion. During this period the urine was collected and its glucose content determined by Benedict's method. The estimation of the blood sugar was by the Kramer-Gittelman<sup>18</sup> method in which blood is obtained by pricking the finger tip. It has been shown by Foster<sup>19</sup> that the sugar content of such blood is identical with that derived from the radial artery, in other words blood from the finger tip may be considered arterial blood. Two days later a similar procedure was carried out. In the interval between the tests parathyroid extract-Collip (Eli Lilly & Co.) was given. The dosage and time of administration are indicated in Table I. Some of the cases received calcium lactate by mouth in addition to the parathyroid extract. An analysis of the results (see Table I) reveals the following:

a. Fasting level.—In the first eight cases the fasting level blood-sugar concentration of the second curve is lower than that of the first, in the last three, however, the reverse is true.

TABLE I

CASE NO	1		2		3		4		5		6		7		8		9		10		11	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Fasting level	177	148	249	161	172	162	243	180	207	160	186	145	259	259	390	50	181	190	219	15	184	20
45 minutes postcubum	333	279	392	271	266	252	360	333	331	241	314	233	444	444	574	359	266	271	351	308	261	310
2 hours postcubum	337	339	423	444	333	317	463	444	317	197	249	224	522	302	670	50	274	299	404	475	279	320
3 hours postcubum	353	327	375	383	337	325	389	380	228	277	186	139	467	290	434	330	199	242	293	265	248	296
Urine glucose for three hour period	10.3	12.8	17.5	21.0	9.6	8.6	-	-	13.4	9.6	13.6	16.4	-	-	31.5	19.0	9.0	12.5	12.7	8.0	6.3	6.3

Urine Glucose expressed in grams  
Blood sugar in milligrams per 100 c.c. of blood

Cases 1 and 2 10 units of parathyroid extract Collip 16 hours before second glucose tolerance test.

Cases 3 and 4 5 units of parathyroid extract Collip 16 hours before second glucose tolerance test.

Cases 5 and 6 10 units of parathyroid extract Collip 16 hours before second glucose tolerance test.

Cases 7 and 8 10 units of parathyroid extract Collip 16 hours before second glucose tolerance test.

Cases 9 and 10 20 grains calcium lactate three times daily in the interval between tests.

Cases 11 and 12 10 units of parathyroid extract Collip 16 hours before second glucose tolerance test.

A = control curve.

B = curve after administration of parathyroid extract.



b This is also true of the forty-five minute postcubum blood-sugar concentration

c In the two hour postcubum blood-sugar concentration one case shows no change, in four, that of the second curve is higher than that of the first, and in six, the reverse is true

d In the three-hour postcubum blood-sugar concentration two cases show no change, in five, that of the second curve is higher than that of the first, and in four, the reverse is true

One is forced to conclude that parathyroid extract does not appreciably influence the blood-sugar curve

e Glycosuria—Three cases show an increase in glycosuria after parathyroid administration, four a decrease, and two no change

#### SUMMARY AND CONCLUSIONS

The literature on the relationship of calcium to diabetes mellitus and of the parathyroids to diabetes and carbohydrate metabolism in general has been revived. One would gather from the work of Cammidge and Howard<sup>13, 14</sup> and others that although by itself parathyroid extract has no influence on carbohydrate metabolism, it distinctly augments the potency of insulin. The administration of glucose causes a secretion of insulin into the circulation. The glucose tolerance test may be considered an index of the amount of insulin secreted. If parathyroid extract increases the potency of insulin, this should make itself evident in a reduction in the height and length of the blood-sugar curve and in the amount of glycosuria. We did not find this to be the case. Our results are completely at variance with those of Cammidge and Howard and others. If we are to assume that parathyroid extract-Collip constitutes the sole active principle of the parathyroids, then we are forced to conclude that the latter are not concerned with carbohydrate metabolism.

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#### REFERENCES

- <sup>1</sup>Wishnofsky, M. Studies in Calcium and Carbohydrate Metabolism. I. Calcium and Glucose Tolerance in Diabetes Mellitus, *Jour. Lab. and Clin. Med.*, 1927, xiii, 133-138.
- <sup>2</sup>Von Morawetz, W. Stoffwechselversuch bei Diabetes Mellitus, *Centralbl. f. inn. Med.*, 1897, xviii, 921-932.
- <sup>3</sup>Von Morawetz, W. Stoffwechselversuch bei Diabetes Mellitus, *Ztschr. f. klin. Med.*, 1898, xxiv, 59-65.
- <sup>4</sup>Von Noorden, C. *Metab. and Pract. Med.*, 1907, iii, 600.
- <sup>5</sup>Falta, W., and Whitney, J. L. Zur Kenntnis des Eiweiss- und Mineralstoffwechsels pankreatischer Hunde, *Beitr. z. chem. Phys. u. Path.*, 1908, i, 224-228.
- <sup>6</sup>Kahn, M., and Kahn, M. H. Lime Deficiency of Diabetes Mellitus, *Arch. Int. Med.*, 1918, xviii, 212-227.
- <sup>7</sup>Davies, D. T., Dickens, F., and Dodds, E. C. Observations on Preparation, Properties, and Source of Parathyroid Hormone, *Biochem. Jour.*, 1921, xv, 695-702.
- <sup>8</sup>Rothchild, F., and Jacobsohn, M. Der Einfluss von Insulin auf die Blutzusammensetzung beim Diabetes Mellitus, *Ztschr. f. klin. Med.*, 1926, civ, 70-79.
- <sup>9</sup>Harrop, G. A., and Benedict, E. M. The Participation of Inorganic Substances in Carbohydrate Metabolism, *Jour. Biol. Chem.*, 1924, lxx, 683-697.
- <sup>10</sup>Collip, J. B. The Calcium Mobilizing Hormone of the Parathyroid Glands, *Jour. Am. Med. Assn.*, 1927, lxxviii, 565.

- <sup>11</sup>Eppinger, Falta and Rudinger Quoted in Barker's Endocrinology and Metabolism, 1922  
1, 635
- <sup>12</sup>Marine Quoted in Barker's Endocrinology and Metabolism 1922 1 635
- <sup>13</sup>Cambridge, P. J. and Howard H. A. H. Interrelation of Insulin and other Gland Ex-  
tracts in Metabolism, Jour Metab Research 1924 vi 189
- <sup>14</sup>Cambridge P. J. and Howard, H. A. H. Influence of Insulin upon Activity of Diastatic  
Ferment of Liver Jour Metab Research 1925 v 95 105
- <sup>15</sup>Winter L. B. and Smith, W. On a Possible Relation between the Pancreas and the  
Parathyroids Jour Physiol 1923 lvi 108 110
- <sup>16</sup>Forrest W. D. Effect of Parathyroid on Blood Sugar Curve After Insulin Brit Med  
Jour, 1923, ii 916 917
- <sup>17</sup>Petty, O. H. Stoae, W. H. and Schniffer H. W. Clinical Application of Parathyroid  
Extract 1926 xlv 173 182
- <sup>18</sup>Kramer B. and Gittelman I. F. Technique for Quantitative Estimation of Glucose in  
Small Amounts of Blood Jour Am Med Assn, 1923 lxxxii 1171
- <sup>19</sup>Foster G. L. Some Comparisons of Blood Sugar Concentration in Venous Blood and in  
Finger Blood, Jour Biol Chem 1923 lv 291 301

544 WILLIAMS AVENUE

## NEW IODO DERIVATIVES OF PHTHALEINS\*

BY FREDERICK R. GREENBAUM D. SC., PHILADELPHIA PA

**H**ALOGENATED phthaleins have recently aroused a great deal of inter-  
est. They have been introduced for cholecystography by E. A. Graham  
and his coworkers.<sup>1</sup> He investigated tetraiodophenolphthalein and tetra-  
bromophenolphthalein. Today tetraiodophenolphthalein as a sodium salt,  
tetrabromophenolphthalein and tetrachlorophenolphthalein are prepared and  
marketed by a number of American manufacturers. They are being used in  
cholecystography.

The very unpleasant side reactions that occur after intravenous injection  
of these compounds show that they are still very toxic and hence a search for  
new iodine compounds in this series of phthaleins was indicated. The iodine  
compounds were preferably synthesized on account of the high atomic weight  
of iodine and for the purpose of cholecystography was absolutely essential  
to make these drugs efficient.

Previous to this time halogenated phthaleins have been considered im-  
portant from two points of view: first as indicators, and second for spectra  
absorption. Tetrabromophenolphthalein and tetrabromofluorescein were  
studied by D. L. Pratt, F. B. Doam and A. W. Harvey.<sup>2</sup>

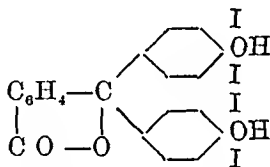
For this investigation the two different isomers of tetraiodophenol  
phthalein, the  $\alpha$  tetraiodophenolphthalein and then the  $\beta$  tetraiodophenol  
phthalein were prepared. Then a completely saturated iodine compound of  
phenolphthalein was synthesized, namely octaiodophenolphthalein, which as  
far as I am aware has not been prepared before. This compound should prove  
of immense interest in cholecystography as it has the highest molecular  
weight of them all. Its exceedingly high content of iodine should prove very  
advantageous in this field.

The introduction of iodine was accomplished by several methods. Phe-  
diodo-dinitro-phenolphthalein, and iodine introduced into dinitrofluorescein  
gave di-iodo dinitro fluorescein. Metacresolphthalein gave diiodo-metacresol-  
phthalein. Rhodamine B gave by one method a mono-iodo rhodamine B, and  
by using another method a diiodo-rhodamine B was obtained. Thymol-  
phthalein gave when treated with iodine a mono-iodothymolphthalein, but no  
diiodo compound could be prepared.

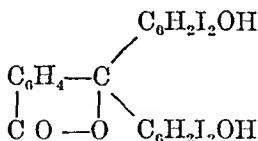
The introduction of iodine was accomplished by several methods. Phe-  
nolphthalein is the phthalein which is the easiest to halogenate as the higher  
homologues often require more energetic methods used than the one in the  
case of phenolphthalein. The iodo compounds obtained were analyzed for  
iodine by the Carius' method.

#### EXPERIMENTAL PART

$\alpha$  tetraiodophenolphthalein of the following structural formula was pre-  
pared by the well-known method of Classen and Loeb.<sup>4</sup>

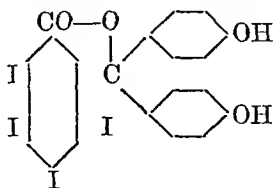


It is an amorphous white powder, insoluble in cold or hot water and in-  
soluble in acids, soluble, however, in hot ethyl alcohol, in ether and other  
organic solvents, and soluble in dilute alkalis with a blue color. This color  
disappears on the addition of more alkali. The compound decomposes at  
220° C giving off vapors of iodine. The compound



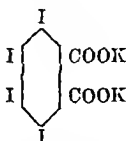
analyzed for iodine gave 61.6 per cent, and calculated to contain 61.8 per  
cent of iodine.

In order to prepare the isomers to the above compound, namely, the  
 $\beta$ -tetraiodophenolphthalein, the structural formula of it looks as follows

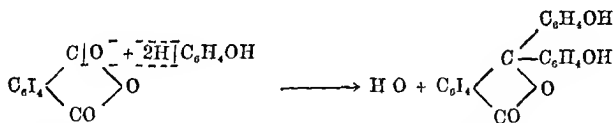


It contains the four atoms of iodine in the phthalic acid ring, while the alpha  
compound contains the iodine in the phenolic rings. It was necessary to  
first prepare tetraiodophthalic acid anhydride, and then to fuse this com-  
pound with phenol to form  $\beta$ -tetraiodophenolphthalein.

*Tetraiodophthalic acid anhydride* was prepared according to DRP 50177, January, 1889 in the following way 20 g of phthalic acid anhydride was dissolved in 480 g of 15 per cent  $\text{SO}_2$  containing fuming sulphuric acid and was heated to  $90^\circ$  to  $100^\circ \text{C}$ . Then 80 g of iodine was gradually added and the temperature raised to  $18^\circ \text{C}$ , and kept there until the reaction was over. It was then allowed to cool, the acid was decanted, washed with sodium bisulphite, filtered and washed with  $\text{SO}_2$  containing water to remove free iodine, and recrystallized from hot glacial acetic acid. The compound obtained represented a yellow prism with a melting point of  $134^\circ \text{C}$ . It is insoluble in water and organic solvents, soluble in hot glacial acetic acid and also soluble in hot potassium or sodium hydroxide under formation of the potassium or sodium salt of tetraiodo phthalic acid of the following formula



In order to prepare now the  $\beta$  tetraiodophenolphthalein, the tetraiodophthalic acid anhydride is fused with phenol in the following manner. 1 mol of tetraiodophthalic acid anhydride and 2 mol of stannic chloride are heated in an oil bath to a temperature of  $140^\circ \text{C}$  for several hours. At first the compounds begin to melt, but soon under the dehydrating influence of stannic chloride a chemical reaction takes place which is evident by the change in color from a light yellow to a pink, to a red and finally to a dark red. A combination occurred according to the following equation



The dark red fusion obtained was extracted after cooling with hot water to dissolve the stannic chloride. The residue is dissolved in warm dilute (15-20 per cent) sodium hydroxide solution and this pink or reddish solution is filtered off from the insoluble portions which consist of unchanged tetraiodophthalic acid anhydride. The filtered solution is acidified with dilute hydrochloric acid, filtered, washed, redissolved in sodium hydroxide, acidified again and filtered, and washed with hot water until free from acid and dried. The analysis gave 57.75 per cent of iodine and calculated, for  $\text{C}_{20}\text{H}_8\text{O}_4\text{I}_4\text{Na}$  — m.w. 866, should be 58.6 per cent of iodine.

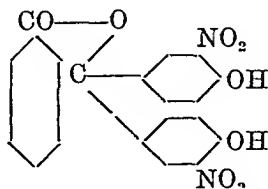
The free  $\beta$  tetraiodophenolphthalein represents an amorphous powder, insoluble in cold or hot water, insoluble in all common organic solvents, but soluble in alkalis with a pink to a reddish color which disappears on addition of strong alkalis. The  $\alpha$  tetraiodophenolphthalein gives a blue color with dilute alkalis.

From this  $\beta$ -tetraiodophenolphthalein, the *octoiodophenolphthalein* was prepared as follows

Twenty gm of  $\beta$ -tetraiodophenolphthalein was dissolved in excess alkali, and to this solution 72 g of iodine which was dissolved in 84 g of 20 per cent sodium hydroxide solution was added under stirring and heated to boiling. Then it was gradually acidified by adding in a fine stream glacial acetic acid, under constant stirring and heated to a boil for one-half hour, after this time it was neutralized with sodium hydroxide solution and then rapidly acidified with hydrochloric acid. It was again heated to a boil, allowing the supernatant solution to settle, decanted, and then filtered off the precipitate and then washed with water until free from acid. The precipitate was redissolved in sodium hydroxide, sodium bisulphite added to the solution and acidified which precipitated the iodo compound and at the same time liberated sulphur dioxide from the sodium bisulphite, this removes any free iodine included in the precipitate of octoiodophenolphthalein. The precipitate was then filtered off, washed thoroughly with water until free from acid or any other impurities, and dried in vacuum desiccator to constant weight. The analysis gave 76.25 per cent for iodine and calculated for  $C_{30}H_{16}O_4I_8$  (m.w. 1326) the iodine content should be 76.60 per cent.

*Detoiodophenolphthalein* is a yellow crystalline substance insoluble in cold or hot water, easily soluble in dilute alkalies, the solution having a faint greenish color. This substance on account of its high molecular weight (1326) and its high content of iodine 76.25 per cent is particularly suitable for experimentation in cholecystography, and the attention of physicians working along these lines should be directed to this new and very interesting iodophthalein.

The next iodophthalein prepared used as a starting material dinitrophenolphthalein. Dinitrophenolphthalein was prepared according to the German patent in the following way: 100 g of phenolphthalein was dissolved or suspended in 100 cc of glacial acetic acid, and is then gradually treated, under cooling and stirring, with a mixture of 70 parts of nitric acid (specific gravity 1.48) and 210 parts of concentrated sulphuric acid, care being taken that the temperature remains at 20° C. The liquid after all the acid has been added gets slightly warm and on cooling and standing a yellow crystalline nitro compound of phenolphthalein is precipitated, which is filtered and washed. It is then recrystallized once from boiling glacial acetic acid and represents dinitrophenolphthalein.



Dinitrophenolphthalein is a yellow crystalline substance, melting point 196° C, insoluble in cold or hot water, soluble in sodium hydroxide with an

orange color, and can be reprecipitated with an acid. It is slightly soluble in ethyl alcohol and methyl alcohol, slightly soluble in ether, easily soluble in hot glacial acetic acid from which it can be recrystallized. The analysis for nitrogen gave 6.75 per cent and 6.93 per cent, and calculated for  $C_{16}H_{10}O_4N$  the nitrogen amounts to 6.86 per cent.

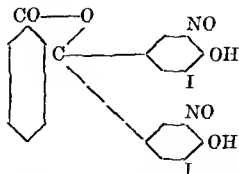
This pure dinitrophenolphthalein was used for the introduction of iodine which resulted in the formation of *diiododinitrophenolphthalein*.

Forty grams of dinitrophenolphthalein was dissolved in 40 g. of 20 per cent sodium hydroxide and 300 g. of water. This solution was boiled and filtered. Then 120 g. of unsublimed metallic iodine was dissolved in 140 g. of 20 per cent NaOH solution and 300 g. of water. The two solutions were now poured together and 125 g. of glacial acetic acid was added slowly under stirring. This addition of acetic acid caused the liberation of iodine in a finely suspended state and the substitution now takes place. It was heated to a boil and kept boiling for about one half hour. Then 85 g. of 20 per cent NaOH solution was added to neutralize the acetic acid and then rapidly acidified with 125 g. concentrated hydrochloric acid and 125 grams of water, boiled for a short time, allowed to settle, the supernatant iodine solution was decanted, the precipitate was washed with hot water, filtered and washed again with water until the wash water was almost colorless. Then the precipitate was dissolved in dilute sodium hydroxide, filtered and reprecipitated with concentrated hydrochloric acid. This precipitate was filtered off and redissolved in NaOH, reprecipitated and washed free from HCl. If necessary, sodium bisulphite was added before acidifying it and then HCl added, this removes the free iodine present. This yellow precipitate was then thoroughly dried on a steam bath and then in a desiccator.

The analysis for nitrogen using the Kjeldahl reduction method and for iodine using Carius' method gave the following results:

calculated for $C_{16}H_{10}O_4N_2I_2$	
for nitrogen	4.2 per cent
found for nitrogen	4.3 per cent
calculated for iodine	38.4 per cent
found for iodine	38.21 per cent

So that the formation of *diiododinitrophenolphthalein* was established, and would have the following structural formula:

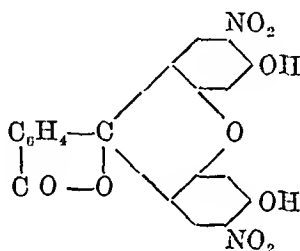


*Diiododinitrophenolphthalein* is a light yellow amorphous powder with a melting point of  $249^{\circ}$  to  $250^{\circ}$  C, insoluble in cold and hot water, soluble in NaOH.

with an orange color, insoluble in the usual organic solvents, soluble in glacial acetic acid. No more iodine can be introduced as all the ortho positions are occupied.

The next higher homolog of phenolphthalein, namely, fluorescein was nitrated according to Beilstein<sup>6</sup>. One part of fluorescein was dissolved in 10 parts of concentrated sulphuric acid, kept at 0° C and 2 parts of concentrated fuming nitric acid was added, then poured on ice and filtered, the precipitate was washed, redissolved in methyl alcohol, filtered and precipitated in H<sub>2</sub>O, filtered and dried.

The analysis for nitrogen gave 6.51 per cent, while calculated for C<sub>20</sub>H<sub>10</sub>O<sub>9</sub>N<sub>2</sub> gave for nitrogen the value 6.6 per cent. So that the dinitrofluorescein of the following formula was obtained.



It represents a yellowish orange powder, soluble in sodium hydroxide with a reddish-blue tint, insoluble in cold or hot water, and insoluble in organic solvents.

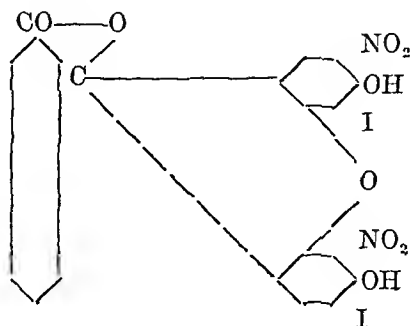
The introduction of iodine into dinitrofluorescein gave a new derivative, e.g., the dinitroiodofluorescein, which was prepared in exactly the same way as the diiododinitrophenolphthalein.

This method of introduction of iodine as described above proved satisfactory in all fluorescein derivatives and in many other phthaleins.

The diiododinitrofluorescein, when analyzed yielded for C<sub>20</sub>H<sub>8</sub>O<sub>9</sub>N<sub>2</sub>I<sub>2</sub>

for nitrogen calculated	4.1 per cent
for nitrogen found	4.1 per cent
for iodine calculated	37.7 per cent
for iodine found	37.1 per cent

So that the following structural formula must be assigned to this compound.

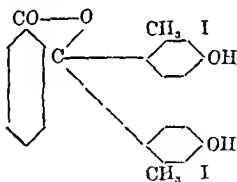


It is an orange red amorphous powder, insoluble in cold and hot water, soluble in ethyl and methyl alcohol, and soluble in ether, soluble in caustic soda solution with a blue color, and on acidification the diiododinitrofluorescein is precipitated

Metaeresolphthalein is a yellowish brown amorphous powder which was prepared by fusing meta cresol and phthalic acid anhydride together in the same way as metaeresolphthalein is prepared according to Beilstein<sup>7</sup> Metaeresolphthalein is soluble in dilute NaOH giving a purple color, and is precipitated with mineral acids. It is soluble in ethyl alcohol and methyl alcohol, and all common organic solvents in concentrated sulphuric acid it dissolves with a brownish color

From metaeresolphthalein an iodo compound was prepared and proved to be *diodometacresolphthalein* in the following manner. 10 g of metaeresolphthalein was dissolved in an excess of caustic soda. This solution was filtered and 20 g of iodine and 20 g of potassium iodide dissolved in 100 cc of water were added gradually under stirring for two hours, acidified with hydrochloric acid filtered and washed redissolved in sodium hydroxide, and reprecipitated with HCl. This procedure was repeated twice, then filtered and washed with water to free from HCl and dried on a steam bath and analyzed

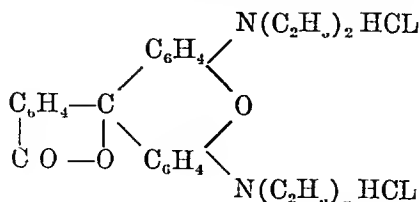
The analysis calculated for  $C_{17}H_{16}O_4I_2$  gave for iodine 42.4 per cent, and the iodine found was 42.0 per cent. Therefore the formula of this compound looks as follows, with a molecular weight of 598



Diodometacresolphthalein is a brown amorphous powder, melting point of 214° C, insoluble in cold and hot water, insoluble in ether somewhat soluble in ethyl and methyl alcohol, very slightly soluble in dilute sodium hydroxide, it dissolves to form a dark brown solution. It is interesting to note that this exhaustive method for the introduction of iodine does not furnish a tetraiodometacresolphthalein, as would be expected, but only a diiodometacresolphthalein in spite of the fact that the four ortho positions are according to the above formula not occupied

The next compound tried was rhodamine B. The commercial compound available is the hydrochloride of tetraethyldiaminophenolphthalein of the following formula

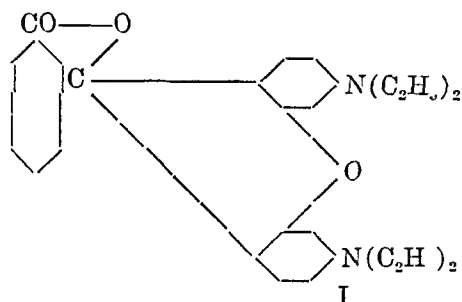




This rhodamine B was treated by two methods for the introduction of iodine. The direct addition of a solution of iodine and potassium iodide to an alkaline solution of rhodamine B resulted in a monoiodorhodamine as the analytical figures show:

calculated for nitrogen	4.90 per cent
found for nitrogen	4.60 per cent
calculated for iodine	23.30 per cent
found for iodine	22.70 per cent

So that the compound obtained was a monoiodo compound of this formula:



It is of course clear that this is an unsaturated compound, but every attempt to introduce more iodine by the use of this method failed. At last the following method was tried and resulted in a diiodorhodamine B. 2.2 g. of highly purified rhodamine B was dissolved in 200 c.c. of water, and to this solution 2.3 g. of iodinetrichloride dissolved in 100 c.c. of water containing 10 c.c. of concentrated hydrochloric acid was added. Immediate precipitation of a reddish flocculent compound occurred. This was filtered off and washed with water until all the acid was removed, then dried and analyzed. The analysis shows:

Calculated for nitrogen (for a diiodo compound)	4.0 per cent
found for nitrogen	3.9 per cent
calculated for iodine	36.4 per cent
found for iodine	36.3 per cent

So this undoubtedly shows that the compound obtained was di-iodo-rhodamine B.

It represented a reddish-brown powder, somewhat soluble in cold water and hot water, insoluble in dilute alkalis, insoluble in acids, very soluble in ether, in methyl and ethyl alcohol. The low solubility of this compound is undoubtedly a disadvantage for medical purposes.

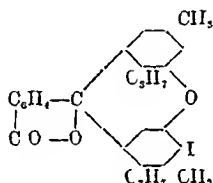
The last phthalein which was included in this study was thymolphthalein. Eight and six-tenths grams of thymolphthalein, obtained from the Eastman Kodak Co., was dissolved in 80 g. of 20 per cent sodium hydroxide solution,

and 60 g of water, boiled, and to this solution 24 grams of iodine which was dissolved in 28 g of 20 per cent sodium hydroxide was added. Then 60 grams of water was added, boiled for half an hour and acetic acid added, boiled again, neutralized with NaOH, and acidified with HCl, boiled, filtered, purified, and dried on a steam bath and in a desiccator.

In spite of this vigorous iodine introduction method only a monoiodo compound resulted, and attempts to use iodine trichloride and iodine monochloride resulted in the same monoiodo compounds, as the analysis shows. For  $C_{12}H_8O_3I$

iodine calculated	23.70 per cent
and for iodine found	23.96 per cent

So that the obtained compound was monoiodothymolphthalein of the following formula



Monoiodothymolphthalein is a brown amorphous powder insoluble in cold or hot water, soluble in common organic solvents, soluble in alkalis with a blue color.

Table I compiles the analytical figures of the prepared iodophthaleins as well as their molecular weights, as the high molecular weight and the iodine content are of the utmost importance for their use in cholelithography.

A glance at this table will show that from the point of view of high content of iodine and high molecular weight the best compound is undoubtedly octoiodophenolphthalein with an iodine content of 76.6 per cent and a molec-

TABLE I  
SHOWING THE ANALYTICAL FIGURES OF PREPARED IODOPHTHALEINS

NAME OF PHTHALEIN	ELEMENTS ANALYZED	THEORY	FOUND	MOLECULAR WEIGHT
$\alpha$ tetraiodophenolphthalein	I	61.8	61.6	822
$\beta$ tetraiodophenolphthalein (Na salt)	I	58.6	57.75	866
Octoiodophenolphthalein	I	76.6	76.25	1326
Diododinitrophenolphthalein	N	4.2	4.3	660
	I	38.4	38.21	
Diododinitrofluorescein	N	4.1	4.1	674
	I	37.7	37.1	
Diodometacresolphthalein	I	42.4	42.0	598
Monoiodorhodamine B	N	4.90	4.60	567
	I	23.30	22.70	
Diodorhodamine B	N	4.0	3.90	694
	I	36.4	36.3	
Monoiodothymolphthalein	I	23.70	23.96	536

ular weight of 1326 Next to this compound are the alpha and beta tetraiodophenolphthaleins, so that a pharmacological study of octoiodophenolphthalein and  $\beta$ -tetraiodophenolphthalein seems highly advisable

#### REFERENCES

- <sup>1</sup>Graham, E A, and Copher, G H Cholecystography, Jour Am Med Assn, 82, May 3, 1924, Cholecystography, Jour Am Med Assn, 84, Jan 3, 1925, Cholecystography, Jour Am Med Assn, 84, April 1925, p 1175
- <sup>2</sup>New and Non Official Remedies 1927 pps 164 167
- <sup>3</sup>Pratt, D L, Doam, F B, and Harvey, A H Jour Am Chem Soc 41, II, p 1293
- <sup>4</sup>Classen, Alex and Loeb, W Berichte der Deutch Chem Ges 28, 1895, II, 1610
- <sup>5</sup>D R P 50177 January 1889, Friedlander, I, p 93
- <sup>6</sup>Beilstein 1896, II, 2060
- <sup>7</sup>Beilstein 1896, II, 1897

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### GINGIVITIS, II

#### THE CLINICAL APPEARANCE OF THE GINGIVAE IN DIFFERENT FORMS AND STAGES\*

BY ROBERT A KEILTY, M D, WASHINGTON, D C

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THE purpose of this paper is to present the physical characteristics of inflammatory changes in the gingivae, as they were observed in the composite picture of a study of 5,000 cases as previously reported<sup>1</sup> My ideas on gingivitis have been embodied in that report, and the present paper is one of a series giving the details of different phases of the subject

The older terms for inflammatory, exudative and ulcerative changes in the gums, such as Ragg's disease and pyorrhea have been discarded in this study The long cumbersome terms, such as "subgingival osteoperiosteal periodontitis" have also been set aside in favor of the shorter and equally inclusive term "gingivitis" Just as the term "pulmonary tuberculosis" includes all forms of tuberculosis in the lung in which the pleura, alveoli, bronchi, and bronchioles are involved and "tonsillitis" all changes on the surface, in the crypts, in the folicles, and in the retrotonsillar solitary glands of the tonsil, so there is included under the term "gingivitis" all degrees of pathology involving the soft structures of the gums from minor to advanced stages including periodontitis or the changes in the tooth socket For the sake of the purist there is no objection to the addition of the term periodontitis when there is x-ray evidence of bone involvement in the chronic cases of gingivitis Such a terminology would then be gingivitis with periodontitis or gingivo-periodontitis This seems unnecessary when it is definitely understood that gingivitis includes all of these advanced changes At any rate pyorrhea should be dropped from the literature It is too firmly fixed to the condition of suppurating gums and loose teeth and can never be made

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\*From the Laboratories of The Diagnostic Center U S Veterans Bureau Washington D C  
Received for publication March 6, 1928

to include the recognition of the early changes in the gums which are far more important in the application of future prophylactic measures

#### THE NORMAL GUMS

The gingivae are soft structures surrounding the necks of the teeth and covering the bony jaws. There is a modified stratified squamous epithelial covering backed up by a corium of loose connective tissue, rich in blood vessels, lymph channels, and submucosal glands. The surface of the gums normally does not join the necks of the teeth at a sharp angle but around each tooth is a small anatomic sulcus. The actual attachment of the gum is slightly below the general surface with the result that a small circular anatomic ditch surrounds each tooth. According to Gottlieb the detachment normally never reaches the cemento enamel junction but is always above it. The reason for its being considered lower before 1920, it seems to me, was due to the fact that pathologic conditions about the gingivae being so numerous, a real histologic normal is rarely seen in the adult.

The union of this surface epithelium at the bottom of gingival pockets, with the periodontal investiture of the tooth and the beginning of the periosteum of the tooth socket, is a very vulnerable point. At the same time this shows a very variable histologic structure, as has just been stated, not because it is variable anatomically but because there are present in most every mouth, even at an early age, pathologic changes at this point which, of course, give variable histologic changes. The interpretation of these changes from the purely histologic standpoint is responsible for much of the discussion in dental literature as to just what constitutes the normal. The interpretation should be made with the pathology as well as the histology in mind. Suffice it to say that the gingival sulcus when present pathologically, offers one of the most vulnerable points in the body protective mechanism and *pari passu*, one of the most frequent portals of entry for invasion with microorganisms.

In the attachment of the perfectly normal gums, which are rarely seen, to the teeth an anatomic sulcus is not grossly apparent. A 22 gauge wire will not catch on any shelf formed by the sulcus nor penetrate any appreciable distance between the gums and the teeth. When this shelf can be seen or a sulcus is present some pathology has taken place.

The gums normally are not red but are quite pallid or slightly pink. There is no boggyiness so that pressure, unlike the case of the lips, does not cause a pallor with a sharp return of capillary blood. While the gingivae in the normal are supplied with a rich capillary network, this is not as apparent grossly as in other mucous membranes of the mouth. The surface of the gums is smooth, not glazed, and presents no granularity. Such normal mouth is occasionally seen but is very rare. Any deviation from this normal constitutes an evidence of pathologic change, the cause of which should be investigated carefully. The best examples of normal gums are seen from 18 to 25 years of age after the trauma of dentition has subsided and before the neglect of teeth has started. On the other hand some of the worst mouths are seen during these ages. It is very rare to see a perfectly normal mouth after the third decade.

## TYPES OF GINGIVITIS

Every mouth is an individual study and subject to conditions peculiar to the individual. No two mouths are exactly alike but in the composite picture certain types of pathologic changes in the gums present definite groups. Gingivitis being an inflammation may be fundamentally divided into acute, subacute, and chronic forms. The specific inflammatory diseases, tuberculosis and syphilis, are not included in this study. The gums are markedly influenced in both of these diseases. This will be the subject of a separate report. All of the phenomena of acute and chronic inflammation of a mucosal surface appear in the gums and contiguous structures and even involve the teeth. Thus rubor, calor, tumor, and dolor constitute the fundamentals of acute gingivitis, and exudation, suppuration, and ulceration represent subacute and chronic gingivitis. While strictly speaking, there is no pathologic entity representing a subacute inflammation, the combination or progression of an acute and chronic inflammation is certainly well seen clinically in the gums.

## ACUTE GINGIVITIS

Acute changes in the gums may be localized to the area about single teeth, when they are often traumatic in origin. These include examples of traumatic occlusion and may or may not be complicated by other etiologic factors, infections, operative procedures, dentures, crowns, etc. The vast majority of acute cases are infectious in origin and involve the entire gingivae both upper and lower, central, lateral right or left, and with degrees of severity. This is exactly the same as in tonsillitis, the involvement here being one tonsil, both tonsils, pillars, fauces or uvula.

In gingivitis the acute process is exudative and necrotic with suppuration and false membrane. This is seen in trench mouth and in the acute flareup of a chronic gingivitis following, for example, the extraction of a tooth. The gums are swollen, tender, red, congested, boggy, pit and blanch on pressure. The anatomic ditches separate from the teeth and are filled with the products of exudation. The slightest touch produces hemorrhage. The teeth are covered with sordes (products of the exudate). The gingival surface, on the fifth to the tenth day of the disease in very severe cases, may be covered with a false membrane which is easily stripped off leaving a bleeding necrotic and ulcerated surface beneath. This often becomes so intensive that the entire gingivae and sides of the cheek, fauces, and tonsils are involved as a stomatitis. The mouth is opened with difficulty and in fatal cases this goes on to cellulitis of the entire face and neck.

All of these changes in degree will be seen in an individual case depending upon the severity of the infection, the immunity reaction of the patient, the time of the infection, and the efficacy of the treatment. Acute gingivitis is accompanied by pain which is often exquisite, and tenderness not only in the gums but the whole face. The patient looks sick and feels bad, the result of systemic infection. The breath is sweetish, offensively foul due to the necrosis and to rapid bacterial reproduction with gas formation. Most of the foul breaths so often attributed to sour stomach and biliousness, if not

due to a decaying tooth, are due to gingivitis. This is proved by the characteristic odors of culture tubes from gingival bacterial flora and by the prompt disappearance of breath odors when adequate local chemotherapeutics is carried out. While this is the picture of "trench mouth," an acute, exudative and suppurative gingivitis, it is not an entity but a part of the general scheme of gingivitis. Acute gingivitis is secondarily divided for classification into traumatic, exudative, suppurative, necrotic ulcerative, and membranous forms.

#### SUBACUTE GINGIVITIS

Subacute gingivitis without a definite pathology does not represent a large group of cases. The group is necessitated by the patients who show subjectively and objectively basic chronic changes with varying degrees of acute flareups. These I believe are due to definite cycles of the infectious organisms involved. These organisms at different periods in their growth represent sexual and asexual stages varying in their toxicity and thus responsible for the acute reactions on the part of the tissue. Reinfection also plays an important part in these subacute cases. The universal custom of kissing and the direct chances for contact is without doubt the *modus operandi* and one of the important reasons for the widespread incidence of gingivitis.

The picture of the gums in subacute gingivitis varies within wide limits. It would seem best to restrict this class to cases with mild pictures of chronic changes. These show localized or focalized tender areas, where the gums are unduly boggy. In these areas the trenches are deep, almost pockets, with heavy exudate while the rest of the mouth is more or less quiescent. This group may also include some of the early insidious chronic cases without any great activity. There are frequently evidences of exudation and infection. These cases are often painless and symptomless and the gums are said to be "healthy red looking." Under the same conditions the acute cases which have subsided and are becoming early chronic may also be classed under the subacute groups. Subacute gingivitis is secondarily divided for classification into exudative, reinfective, focalizing and early chronic.

#### CHRONIC GINGIVITIS

The group under the classification of chronic gingivitis is by far the largest and most of us fall into it. Over 90 per cent of the 5,000 cases studied presented some form of gingivitis and this represents, I believe, a cross section, at least, of the people of this country. The large group of cases studied were mostly residents in the central part of Pennsylvania. During the study I was a little fearful that the results might be a more or less local state of affairs, but in the past eight months I have seen several hundred cases in Washington, D. C., and have found exactly the same conditions and incidence of occurrence. The Washington cases represent a more selected group having had excellent dental care in spite of which gingivitis had continued to progress.

Chronic gingivitis presents a picture much more varied than either of the other two forms. This is due almost entirely to the stage of progression of the disease. It would seem well to first divide them into groups of mild, moderate and advanced types. There is no sharp line between these cases,

all show some of the fundamental changes, but the question of severity of these fundamental changes, length of time they have developed, and the amount of deep bone change, determine then grouping. The question of the bacteriologic flora in a given case plays a very important rôle in the study of that individual case and this relationship will be discussed in another paper. So far we have been unable to classify gingivitis on a basis of its different types of bacterial infection and protozoal infestation, but accumulation of data may provide in the future a more definite classification from this point of view.

All cases of chronic gingivitis should have a complete dental x-ray and those showing periodontal changes about one or more teeth (now almost universally diagnosed as pyorrhea) should be classed as advanced chronic gingivitis or gingivoperiodontitis. All such deep changes will be accompanied by marked changes in the soft structures. The reverse is not true. Severe and advanced chronic changes may appear in the gingivae without x-ray evidence of deep periodontal change.

The surface of the gums in chronic gingivitis is rarely smooth and glistening but is usually glazed and granular. This granularity is often very marked. Some gingivae have little blood vessel reaction, especially the long-standing chronic fibrous forms and are pallid white, others vary through pink and red to deep purplish blue. I have seen the blueness of passive congestion so marked that chronic lead poisoning had been suspected. All chronic cases have some degree of swelling, not the boggy edema of the acute cases but the firmer, more tense edema of passive congestion. Under appropriate treatment gums may subside over a half their thickness. This swelling is mostly fluid but in the advanced cases much cellular reaction with fibrosis of the subgingival mucosa has resulted. Some of the moderate cases and many of the long-standing cases show a ridging along the gingival margins often associated with recession so that the ridge is a half moon. This is always a subgingival fibrosis and the result of organization fibrogenic reaction against infection.

Gingival recession occurs to some degree even in the mild cases but more often is a part of the advanced changes. It is due to the loss of structure by ulceration and equally as important to the contraction following efforts at repair. All of the successfully treated cases will show more recession after treatment than before. The elimination of sulci physically results in retraction of the overlapping gingivae and exposure of dentine. Such patients will often consider that they are worse, the exposed dentine is more tender, the gums have receded and more of the teeth are exposed. They may even say their teeth are elongating or coming out. It is well to explain before treatment that this is an evidence of repair and that in the end no pain or discomfort will result.

Recession may affect a single tooth, a series of teeth as the lower centrals or may be more or less general. It usually involves the labial and lingual surfaces, leaving pyramids of gingival structure between the teeth. Occasionally the reverse is true, marked recession occurring between the teeth with little on the labial and lingual surfaces. This is more apt to be present

in subacute and chronic cases following a destructive, necrotic acute ulcerative gingivitis. Finally recession unassociated with other evidences of gingivitis appears in a small number of cases of tuberculosis and syphilis.

I have stated that the normal gingiva has no macroscopic sulcus or trench at its attachment to the enamel of the tooth which in the normal, as pointed out by Gottlieb, is above the cemento enamel line. The anatomic ditch is therefore an evidence of gingivitis and where present if only one or two mm in depth will show some exudate. The same fact may be stated in another way, the presence of actual exudate no matter how small in amount, in a gingival sulcus is evidence of chronic gingivitis. The so called anatomic ditch or gingival sulcus in chronic gingivitis varies in depth from one or two mm to over one cm where it extends sometimes by direct communication below the root tip of a tooth.

In mild and moderate cases of chronic gingivitis, the sulci are not very deep, usually not involving the periodontal areas, except possibly about one tooth often in the molar areas where for one reason or another the process has become advanced.

The sulci are, generally speaking about evenly divided about most of the teeth. The more shallow sulci are about the centrals, growing deeper toward the molars. The depth of the pockets increases with the progression of the disease, and the generally deep sulci are a part of the advanced cases.

With the development of sulci in chronic gingivitis exudation from and ulceration of the gingival mucous membrane and submucous connective tissue takes place. The character of this exudate varies within wide limits and will be discussed under the phase of bacterial flora. *It is not necessary to express pus from the margins of the gums to make a diagnosis of chronic gingivitis.* The presence or absence of pus is dependent on several factors and many cases of apparently complete absence of gross pus show a gingival exudate that is microscopically teeming with infection.

One of the main purposes in this study is to call especial attention to the early stages of chronic gingivitis and to urge their recognition. *Intelligent prophylaxis in the early cases will do much to save a future trail of diseases, not the least of which are those of the heart and rheumatic group, and ranks in importance with prophylaxis against the progression of tuberculosis and cancer.*

The patients suffering with chronic advanced gingivitis are usually aware of it. The salty, disagreeable taste of pus, the bad breath odor, the bleeding in the gums, the looseness or loss of teeth are all quite manifest. They have in addition, usually been told by their dentists that they have "pyorrhea" and many have had several courses of treatments of the various types now in vogue. In spite of all their care their "pyorrhea" is progressing and they have or have not reached the stage where extraction is necessary.

Chronic gingivitis is secondarily divided for purposes of classification into mild, moderate and advanced as far as the degree is concerned. Exudation, ulceration, recession, and fibrosis cover the gingival pathology while gingivitis with periodontitis may be used to cover the cases with x ray evidence of osseous destruction.



## CONCLUSIONS

It is hoped that, in reporting the results of my observations on studies in mouth infections made during the past several years, that the atmosphere will be somewhat clarified and not thrown into any greater confusion as far as the gums are concerned

The problem of gingivitis as studied grew out of the relationship of mouth infections as etiologic foci in thyroid diseases

The study of gingivitis has reached certain conclusions that would seem to warrant their detailed report at this time, realizing that an open mind should still be held until this troublesome condition is conquered

Critical observations of the gums as to appearance have led to the conclusion that the normal gingiva may be said to be almost rare in adults

The histologic conceptions of the gums in the past must have been based upon the results of pathologic changes and not the perfectly normal relationship

The histologic anatomist must definitely decide just what constitutes the normal gingival sulcus, and the exact relationship between the soft tissue cells and the enamel of the tooth. This is obviously quite difficult for lack of material from the human mouth

From the present study it is concluded that any gross evidence of a sulcus associated with exudate, constitutes an early pathology which breaks a line of defense allowing a beautiful culture bed for microorganisms and protozoa. *This initiates a portal of entry which is believed much more frequent and important than root canals*

A classification of inflammatory changes in the gums for purposes of study and treatment is offered in this presentation. This is based upon the appearances, clinical courses, and pathologic reactions

The character of the bacteriologic flora and protozoal infestation of the gingiva, sulci, and periodontal osseous structures is most important in the whole study, but so far their relationship cannot be offered as a means of classification of different types of gingivitis

Gingivitis is worthy of a continued careful and critical study of its complex phases and the laboratory can accomplish a great deal as fixing much of haziness and controversy concerning it

## REFERENCES

1. Kilty, Robert A. The Present State of Our Knowledge of Gingivitis, *JOUR LAB AND CLIN MED*, 1928, III, No 5, p 451

## A CASE SHOWING UNUSUAL WASSERMANN REACTIONS\*

By JAMES J. SHORT, M.D., and MARGARET F. KELLEY, A.M., New York City

ALTHOUGH in the overwhelming majority of instances the Wassermann test gives reliable and consistent results, there is an occasional rare instance when results obtained are wholly unreliable. Tabulated results with such a case thoroughly studied in several laboratories are set forth in Table I.

### REPORT OF CASE

The patient, S. K., female, aged nineteen, was advised to have a Wassermann test because of a paronychia on her finger. The serum was first examined at the Laboratories of the Life Extension Institute and gave complete fixation with each of two antigens. These antigens were obtained from the New York State Department of Health at Albany; one was the plain alcoholic extract of beef's heart, the other the same cholesterolized. Further tests were made when the patient reported a negative result obtained a few days later from the Laboratory of the New York City Department of Health. McNeil's antigens mentioned in the table were obtained from the National Pathological Laboratory and were prepared by extracting hog's heart several times with ether, desiccating and making up an alcoholic extract from the residue. To this, 0.2 per cent cholesterol was added to form the cholesterolized antigen.

Ordinarily, very good agreement between the Board of Health and McNeil antigens has been obtained.

The laboratories indicated in the table to which specimens were sent are New York State Department of Health, Albany, New York City Department of Health, Mr. John Koopman, Director, 505 Pearl Street, National Pathological Laboratory, Dr. Archibald McNeil, Director, 18 East Forty First Street, Willard Parker Hospital, Sixteenth Street and East River (tests made under supervision of Miss Minnie Wilson) and Laboratory of the late Dr. John A. Fordyce, 8 West Seventy Seventh Street.

It will be observed from a study of the table that not only was there disagreement between different laboratories, but in several instances varying results in the same laboratory. Varying the fixation period slightly frequently changed the result in our experience. There was a tendency in several tests with the McNeil antigen for the alcoholic antigen to hold more completely than the cholesterolized.

### COMMENT

We can offer no explanation for the results obtained in this case other than to say that such instances do occasionally occur. It is questionable if this patient was really syphilitic as her condition would not justify such a diagnosis clinically. Unfortunately we have not been able to follow this patient since these studies were made.

\*From the Laboratories of the Life Extension Institute, New York.  
Received for publication March 13, 1928.

# LABORATORY METHODS

## A NEW ALDEHYDE REAGENT\*

By ROBERT D. BARNARD S.B., CHICAGO, ILLINOIS

THE rôle of acetaldehyde in intermediary carbohydrate metabolism<sup>1</sup> and in the fermentation of various sugars makes it desirable to secure a reagent for the quantitative estimation of this substance in distillates from biologic materials. The reagent problem has been beset by many difficulties since there may be a concurrence of volatile substances which are closely allied to acetaldehyde chemically and may, therefore, introduce a considerable error. In this connection, attention is directed particularly to acetone which gives a positive Schiff reaction, forms a bisulphite addition compound, a cyanhydrin, a hydrazone, and an oxime just as does aldehyde. The method of Bougault and Gros<sup>2</sup> has been found by Chaikoff and Gee<sup>3</sup> to require an acetone correction factor. The ratio of acetone to aldehyde is in some instances greatly in favor of the former so it may readily be seen that correction for the amount of ketone present may be unsatisfactory.

It was felt that any improvement upon the reagents in use should embody the following points:

1. The reagent should be selective or specific.
2. It should be sensitive and allow estimation of small quantities.
3. The reaction should take place at room temperature and be fairly rapid to minimize volatilization and consequent loss of the aldehyde.

With these three points in mind the investigation (the results of which are herein reported) was undertaken. A reagent has been developed which appears to satisfy all the conditions imposed.

### REAGENT

The reagent is a solution of acid fuchsin (Fuchsin S) decolorized by hydrazine hydroxide. It is prepared by adding 75 cc. of 2 per cent acid fuchsin† to one liter of 0.042 per cent hydrazine hydroxide in water which had been boiled to reduce its carbon dioxide content. (The latter solution can be conveniently made up by diluting Eastman 42 per cent hydrazine hydroxide 1:1000.) Decolorization is complete at the end of two hours after which the reagent is ready for use. It has a faint brownish tint which is negligible in the Coleman Bell fuchsin but may be somewhat troublesome in some of the lots prepared by the National Anilin Works. The reagent has been kept for two months at ice box temperature without deterioration.

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A similar case was recently seen by one of us (J J S). The blood of a young lady suffering with urticaria gave complete fixation with the Board of Health antigens on refrigerating overnight in the ice box. Subsequent specimens were all uniformly negative even after provocative treatment.

McNeil<sup>1</sup> reports an occasional rather strong nonspecific fixation in patients having foci of infection especially with streptococci. It has been our observation in several thousand cases that weak nonspecific fixation (plus minus) is frequently obtained with cholesterolized antigens in sera from patients giving histories of previous gonorrheal infections. This happens even when no active gonorrheal focus can be found by either clinical or laboratory investigation.

#### CONCLUSION

While these results show the need for caution in arriving at a diagnosis of syphilis solely from laboratory testimony, it is well to keep in mind that after all the Wassermann test is one of the most accurate and dependable aids in diagnosis that we have. Certainly the ratio of error in this test is far lower than that in the physical examination of the heart and lungs, routine measures which are considered of high value by all clinicians.

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### THE USE OF POSTERS AS A METHOD OF INSTRUCTION IN A DIABETIC CLINIC\*

By JOHN R. WILLIAMS, M.D., AND MARION VYE, B.S. ROCHESTER, N. Y.

THE value of personal instruction to diabetic patients is more and more realized by those who have to deal intensively with the problem. Indeed, it is our belief based on extended experience that the teaching of the patient is of equal importance with clinical and laboratory study of the disease, and that treatment will fail if those afflicted with diabetes do not fairly well understand the principles of dietary control, insulin therapy and personal hygiene.

While most clinicians are in accord with these views there is considerable variance in practice. Perhaps the most common is to give the patient one of the many well known manuals on the market and to supplement this with an occasional talk or demonstration by the dietitian. In our experience, textbooks are too technical, too difficult, and quite likely to be misunderstood, unless elucidated and interpreted by some one well versed in the subject.

The diabetic patient on admission to our clinic is given a loose leaf book made up of mimeographed lessons or instructions. At his first class he is taught in simple language principles of food chemistry, metabolism, diabetes, and insulin action. The topics are presented in orderly manner, and the substance of what is said is printed and numbered in the lesson in his in-

<sup>1</sup>Private communication.

\*Presented before the Hospital Dietetic Council at the Convention of the American Hospital Association, October 1927.

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Fig. 1—Group of colored posters made by cutting out and remounting pictures from well-known advertisements in current magazines. These are then given legends which have a special significance to the diabetic patient.



Fig. 1.—Practically every problem in the daily life of the diabetic patient is illustrated by these posters



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THE rôle of acetaldehyde in intermediary carbohydrate metabolism<sup>1</sup> and in the fermentation of various sugars<sup>2</sup> makes it desirable to secure a reagent for the quantitative estimation of this substance in distillates from biologic materials. The reagent problem has been beset by many difficulties since there may be a concurrence of volatile substances which are closely allied to acetaldehyde chemically and may, therefore, introduce a considerable error. In this connection, attention is directed particularly to acetone which gives a positive Schiff reaction, forms a bisulphite addition compound, a cyanhydrin, a hydrazone, and an oxime just as does aldehyde. The method of Bougault and Gros<sup>3</sup> has been found by Chaikoff and Gee<sup>4</sup> to require an acetone correction factor. The ratio of acetone to aldehyde is in some instances greatly in favor of the former so it may readily be seen that correction for the amount of ketone present may be unsatisfactory.

It was felt that any improvement upon the reagents in use should embody the following points:

- 1 The reagent should be selective or specific.
- 2 It should be sensitive and allow estimation of small quantities.
- 3 The reaction should take place at room temperature and be fairly rapid to minimize volatilization and consequent loss of the aldehyde.

With these three points in mind the investigation (the results of which are herein reported) was undertaken. A reagent has been developed which appears to satisfy all the conditions imposed.

### REAGENT

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tients in our clinic. In one day's lesson we endeavor to group all the common foods which are safe and useful in their order of comparative safety. In another group we show foods which are dangerous and forbidden. In other groups we illustrate ideas about exercise, bathing, tobacco, music, marriage, personal hygiene, the care of the feet, and so on. We do not rely entirely on this method of teaching but we use it merely as supplemental to class talks and demonstrations. Sometimes one poster will provoke a discussion among the patients and will stimulate interest and original thinking with them. We have found it helpful, in the training of little children, to adapt the same idea to scrapbook use. We have the child make such a book in which he pastes pictures of all the foods that are safe for him to use and those which he must avoid. We endeavor to see that he has a pictorial representation of every fact which is important for him to understand. We try thus to illustrate his daily life from the moment when he arises until he goes to bed at night. We give the child a set of can labels and encourage him to search through magazines for pictures which will be of service. Sometimes it becomes a family game, of more practical use and just as much fun as cross word puzzling.

In conclusion, we present a poster method of instructing diabetic patients, which we use to supplement the older and well understood methods of teaching. In our hands it has been of value.

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## REACTION

After addition of aldehyde or acetone in the cold, the reagent turns pink. It does not reproduce the exact shade of the original fuchsin but the color has an orange cast. The time elements are important for these two substances the color developing gradually and reaching a maximum in the case of acetaldehyde in one to fifteen minutes in the case of acetone in one to three hours in both cases depending on the concentrations. Acetone must be present in great excess to give rise to the color within one hour and since the aldehyde reacts with such relative rapidity it has been found possible to determine 0.0001 gm. of acetaldehyde in the presence of 0.1 gm. of acetone. The color due to the action of aldehyde upon the reagent fades within a few hours if the reaction mixture be exposed to air when the reaction mixture is stoppered it has been observed to persist for at least a week. The color produced by acetone has been observed not to fade for at least a week both in stoppered and unstoppered mixtures.

## INTERFERING SUBSTANCES AND CONDITIONS

Free acids, when added to the reagent produce in it a color identical with pure acid fuchsin solutions. Their action is immediate which will distinguish them from aldehyde. The color produced by addition of acid further more, is fully twice the depth obtained by either aldehyde or acetone and contains no element of orange.

Oxidizing agents such as molecular iodine, potassium ferricyanide, sodium persulphate and potassium chromate and the salts of the heavy metals behave toward the reagent in a manner similar to free acids with the additional phenomenon of effervescence. In the cases of silver, copper or mercury salts the free metal is precipitated.

Bases or strong reducing agents will prevent the formation of the color by acetaldehyde and by acetone.

The color reaction with aldehyde will not take place in buffered solution.

## METHOD OF APPLICATION

The distillate in which the quantity of aldehyde is to be determined should be unbuffered, neutral or alkaline to litmus and acid to phenolphthalein. One c.c. of the reagent is added to one c.c. of the distillate in the colorimeter cup and mixing effected by moving the plunger up and down through the solution three or four times. The color is compared at the end of fifteen minutes with a standard made simultaneously and in the same manner but using one c.c. of a stock solution of aldehyde containing 0.1 gram per liter\*. These directions are for the Leitz micro colorimeter which will accommodate 2 c.c. of solution and the working range for the amounts of aldehyde and reagent given is between 0.02 mg. and 0.14 mg. For higher concentrations the distillate must be diluted to a point where the aldehyde content falls within this range.

\* Since acetaldehyde upon standing often develops some free acid which may interfere it has been our custom to add one or two drops of 1 per cent sodium bicarbonate to the liter of stock solution.

## DISCUSSION

As a colorimetric reagent, the one here presented offers some disadvantages. The color where small quantities of aldehyde are involved may be very faint and entirely unsuited for reading. The bi-color nature of the reaction mixture has made it impossible to use a permanent standard. The range has narrow limits.

The reagent is sensitive to one part of aldehyde in one hundred thousand, the reaction takes place at room temperatures and a determination may be effected in fifteen minutes.

## SUMMARY

A new quantitative reagent for acetaldehyde in the presence of an excess of acetone is described.

The author extends his heartiest thanks to Mr R D Templeton and Dr A J Carlson for their cooperation in ascertaining the quantitative usefulness of the reagent.

## REFERENCES

- <sup>1</sup>Neuberg, C. Ber Chem Ges, 1922, li, B, 3624
- <sup>2</sup>Peterson, W H, and Fred, E B. Jour Biol Chem, 1920, lvi, 29
- <sup>3</sup>Bougault, J, and Gros, R. Jour Pharmacol et chem, 1922, vii, 265
- <sup>4</sup>Gee, A H, and Chaikoff, I L. Jour Biol Chem, 1926, lxx, 151

## DOUCHE AND SHOWER AND SPECIAL CABINET FOR GASTRO- INTESTINAL TUBES\*

BY MOSES EINHORN, M D, NEW YORK

THE recent rapid increase in the use of Ewald and duodenal tubes for obtaining gastric content after Ewald or fractional test meals and for the performance of biliary drainage has created the need for an apparatus which would solve the problem of the cleansing and storing of the tubes used in these operations. The importance of using absolutely clean tubes in order to obtain the best results cannot be overemphasized. The technician must be sure that the insides of the tubes which he uses are free from any remnants of previous examinations, otherwise the results he obtains will be open to question, since the particles remaining from previous examinations will influence the results obtained in later tests. Secondly, since it is impossible to use a new tube for each patient, it is necessary to take every precaution to prevent these tubes from being the agencies for the spread of contagious diseases by administering a thorough cleansing before and after each examination. The length of these tubes together with other intrinsic difficulties, particularly in the case of the narrow duodenal tubes, has made the problem of cleaning and storing them a difficult one, and it is in the hope that my colleagues will find my

\*Received for publication February 26 1928

apparatus as convenient and efficient a solution of the problem as I have found it to be that I present the apparatus described below

Two extensions, each of which contains a stopcock, are attached respectively to the back of the hot and cold water faucets of a sink. A union connects these extensions and from the middle of this union an arm projects. To this projection, the actual apparatus is attached (see Fig 1). The apparatus consists of a long tube on the bottom of which three specially devised faucets are attached, four inches apart. These faucets terminate in a disk the center of which contains an outlet into which the standard hard rubber Ewald connection fits exactly. The remainder of the tube is perforated in such fashion

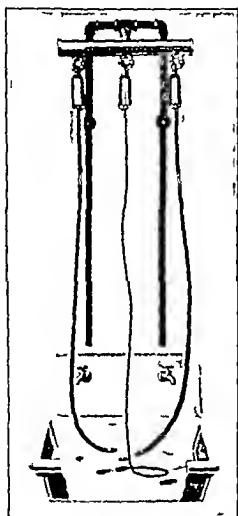


FIG 1

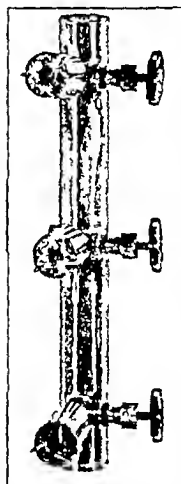


FIG 2

that the shower of water emerging therefrom strikes against and completely envelopes the outside of the attached tube. A clip has been devised which permits of the insertion or withdrawal of the tube with a single motion of but one hand (see Fig 2). The reverse end of a standard hard rubber Ewald connection is narrowed so that it fits into a duodenal tube. By the use of this slightly altered connection piece, this apparatus cleans duodenal tubes in the same fashion as Ewald tubes. The number of outlets may be increased when a greater number of tubes are to be cleaned.

One of the most attractive features of this apparatus is the extreme simplicity of its operation since it requires no attention or effort upon the part of the technician. The sink faucets are closed and the stopcocks in the extension are opened according to whether the tubes are to be cleaned with

hot, lukewarm, or cold water. The tubes are then inserted in the special faucets. The water will now flow into the central tube of the apparatus and by opening the stopcocks on this tube one or all of the tubes may be cleaned. Once the water is turned on and the tubes are inserted, the technician is free to do other work until he is satisfied that the tubes have been thoroughly cleaned.

A special cabinet has been constructed for the storing and drying of these tubes (see Fig 3). A bar across the top of this cabinet is equipped with clips of the type previously described. Since the cabinet is almost five feet high,

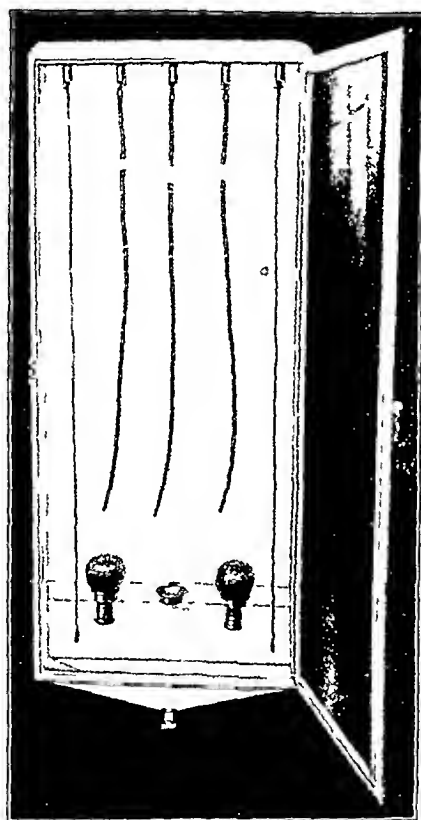


Fig 3

the tubes are able to hang vertically when they are inserted. The Ewald bulbs may be stored in holders which are provided for that purpose. The floor of the cabinet is made in such fashion as to permit drainage of the accumulating moisture into a central outlet which is regulated by a stopcock. Numerous perforations in the sides and top of the cabinet supply a rapid circulation of air which causes the tubes to be dried quickly. The cabinet is provided with a glass paned door which, when shut, acts to prevent the entrance of dust.

I have observed that the following advantages are obtained by using the above described apparatus.

- 1 It is now possible to clean the tubes with water of any temperature
- 2 The tubes are now thoroughly cleaned both on the inside and outside
- 3 The technician can save time by cleaning many tubes in a single operation instead of cleaning each one separately as was formerly necessary
- 4 Except for the insertion of the tubes and the turning on of the water the above described method of cleaning the tubes requires no attention or effort on the part of the technician
- 5 The tubes are more thoroughly cleaned than previously since the water descends from a great height and consequently has sufficient power to remove all the particles from the inside and outside walls of the tubes
- 6 Since the tubes hang vertically in the cabinet, folding or misshaping of the tubes is prevented
- 7 The cabinet enables the tubes to be kept clean and sanitary

983 PARK AVENUE

## THE CLINICAL VALUE OF THE SPINAL FLUID TEST OF TAKATA AND ARA\*

BY B. L. MONIAS, PH.D., CHICAGO

THE difficulties encountered sometimes in preparing a satisfactory colloidal gold solution for the Lange's test have led to the device of more simple reactions which can be performed also in small laboratories or in the physician's office. None of these reactions, however, has so far been able to replace the Lange's test. Recently Takata and Ara<sup>1</sup> have reported on good results with a simple reaction which is based on the following principle. By mixing a mercuric chloride solution with sodium carbonate in the presence of the normal spinal fluid a colloidal solution of mercuric oxide is formed which turns a deep bluish violet by adding a solution of diamond fuchsin. With pathologic spinal fluids the reaction is different. The abnormal protein content of the latter causes either a flocculation of the mercuric oxide with a discoloration of the fluid or color changes from bluish violet to pink with practically no precipitation. The first type of reaction according to Takata and Ara is typical of syphilis of the central nervous system while the change of the color occurs in bacterial meningitis. These observations have been confirmed during the last year by several German investigators notably Blum, Jacobsthal and Joel,<sup>2</sup> Hantsch,<sup>3</sup> Knigge,<sup>4</sup> and Nicole.<sup>5</sup>

I have used the Takata-Ara test in connection with the other spinal fluid reactions on over one hundred spinal fluids and since the results have been very satisfactory I feel justified in making the American laboratory workers familiar with it.

\*From the Uhlen Memorial Laboratory of the Grant Hospital of Chicago.  
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## THE TECHNIC OF THE REACTION

The following solutions are required

- 1 A 0.3 per cent solution of sodium chloride
- 2 A 10 per cent solution of sodium carbonate This solution is recommended by Takata and Ara but can be replaced by a N/10 sodium hydroxide solution of which the double amount is taken In cases of meningitis the color change is more distinct with the sodium hydroxide than with the carbonate
- 3 A 0.5 per cent solution of mercuric chloride
- 4 A 0.02 per cent solution of diamond fuchsin in distilled water

The spinal fluid must be free from blood and should not be kept longer than twenty-four hours before making the test The test A series of 10 standard Wassermann test tubes are set up and one c.c. of the 0.3 per cent sodium chloride solution is placed in the second tube and up to the tenth tube One c.c. of the spinal fluid is placed in the first and second tubes and after mixing, one c.c. is withdrawn from the second tube and filled in the third tube, and this procedure is repeated with the following eight tubes The last c.c. of the dilution is discarded There is now a range in the dilution from the concentrated spinal fluid in the first tube to 1/512 in the last tube One drop from a capillary pipette of the sodium carbonate or two drops of the sodium hydroxide solution are added to each tube and mixed well The test is completed by the addition of the Takata Ara reagent This reagent consists of equal parts of the mercuric chloride and fuchsin solutions mixed immediately before using The solutions of mercuric chloride and fuchsin keep indefinitely Three-tenths c.c. are used in each tube The test tubes are shaken thoroughly and kept at room temperature Takata and Ara first recommended three readings, namely, after fifteen minutes, thirty minutes, and twenty-four hours In a later publication Takata said that only one reading should be taken after twenty-four hours From my experience I would say that some information can be obtained after thirty minutes and that the result becomes final after twelve hours

Two different forms of reactions may take place with pathologic spinal fluids First, the mercuric oxide flocculates, absorbing the dye The strongest reaction causes a complete discoloration of the fluid and large, deep blue precipitates settle at the bottom of the tube If the reaction is less complete, the supernatant fluid remains more or less blue and the sediment is finer The intensity of the flocculation is recorded in the following way (+), +, +<sup>1</sup>, +<sup>2</sup>, +<sup>3</sup>

Second, the color of the solution changes from bluish-violet to reddish-violet, purple or pink The fluid remains clear or becomes cloudy This result is indicated by a circle O reddish-violet, O<sup>1</sup> purple, O<sup>2</sup> pink If precipitation and color change occur simultaneously the two signs are combined, e.g., O<sup>1</sup>+<sup>2</sup> indicating a marked precipitation with a change of the color to purple

## RESULTS

A *Normal Spinal Fluids*—Five fluids have been selected to demonstrate the reaction. The solution has a deep bluish violet color and remains so. There is sometimes a slight precipitation in the last three tubes which is of no significance. I suggest, therefore, that the last three tubes with the highest dilutions be omitted.

TABLE I  
NORMAL SPINAL FLUIDS

NO	WASSERMANN	KAHN	LANDE	NONNE	TAKATA ARA
1	neg	neg	neg	neg	-----
2	neg	neg	neg	neg	----- (+)
3	neg	neg	neg	neg	----- + + +
4	neg	neg	neg	neg	-----
5	neg	neg	neg	neg	----- (+) + +

TABLE II  
B SYPHILITIC SPINAL FLUIDS

NO	CLINICAL DIAGNOSIS	WASSERMANN		KAHN		NONNE	LANDE	TAKATA ARA
		BLOOD	SPINAL FLUID	BLOOD	SPINAL FLUID			
1	General							
2	paresis	++++	++++	++++	++++	+	355543200000	+ + + + + (+) - - - -
3	"	+++	++++	+++	++++	+	555531000000	+ + + - - - - -
4	"	++	+++	++	+++	++	555531000000	+ + + - - - - -
5	"	++++	++++	++++	++++	++	210000000000	+ - - - - - - -
6	"	++++	++++	++++	++++	++	421000000000	+ - - - - - - -
7	"	++++	++++	++	+++	++	554444200000	+ + + + + - - - -
8	"	++++	++++	++++	++++	++	555554200000	+ + + + + + + + -
9	"	++	+++	+++	+++	+	555531000000	(O+ ) + + - - - -
10	"	++	++	++	++	+	354431000000	+ + - - - - - -
11	"	++++	++++	++++	++++	+	555443210000	+ + + + + - - - -
12	"	++++	++++	++++	++++	+	554433210000	+ + + + + - - - -
13	"	++	++	++++	++++	++	554443210000	+ + + - - - - -
14	"	++++	++++	++++	++++	++	555443210000	+ + + - - - - -
15	"	++++	++++	++++	++++	++	555554433210	(O+ ) + + + + + - - -
16	Tabs							
17	dorsalis	+	+	++	+	+	123321100000	+ + (+) - - - - -
18	"	neg	+	neg	+	+	001221000000	+ (+) - - - - -
19	"	++	++++	++	+++	+	112211000000	- - - - - - - -
20	Syphilis	++++	++++	++++	++++	+	224555530000	+ + + + + (+) - - - -
21	of CNS							
22	"	+++	++	+++	++	+	112332100000	+ + + - - - - -
23	"	+	++	++	+	±	223321000000	+ + - - - - - -

The strongest reactions are obtained in general paresis. Complete flocculation takes place constantly in the first tube and also often in the second, third and sometimes in the fourth and fifth tubes. The precipitate is stained a deep blue. In two instances the color in the first tube had changed to purple.

In syphilis of the central nervous system the reaction is similar to that in general paresis. A differentiation between a parietic and a syphilitic curve is not possible. In tabs dorsalis the reaction is less distinct or negative.

All of the eight cases showed a very distinct change of the color to pink in the first tube. The other tubes remained almost unaffected with the tuberculous spinal fluid while in epidemic meningitis, the content of the second

TABLE III  
C MENINGITIS

NO	CLINICAL DIAGNOSIS	NONNE	LANGE	TAKATA ARA
1	Tuberculous meningitis	++++	000011321000	O <sup>2</sup> O <sup>1</sup> -----
2	" "	+	000111000000	O - -----
3	" "	++++	000123100000	O <sup>2</sup> O -----
4	Streptococci meningitis	++	001222210000	O <sup>2</sup> - -----
5	Epidemic meningitis	++++	000022110000	O <sup>2</sup> O <sup>2</sup> -----
6	" "	++++	000012210000	O <sup>2</sup> O <sup>2</sup> -----
7	" "	+++	000011100000	O <sup>2</sup> O <sup>2</sup> O -----
8	" "	+++	000000111100	O <sup>2</sup> O <sup>1</sup> O -----

and sometimes also of the third tube assumed a pink coloration. In none of these cases was there any precipitation, although the fluid in two instances appeared slightly cloudy.

#### SUMMARY

The Takata Aia test is based upon the formation in normal spinal fluids of colloidal mercuric oxide when mercuric chloride and sodium carbonate or sodium hydroxide are added. Fuchsin serves as an indicator and stains the fluid a bluish violet.

Pathologic spinal fluids either produce a precipitation of the mercury with absorption of the dye or a change of the color to purple or pink.

The flocculation is characteristic of syphilis while the change of the color is observed in bacterial meningitis.

The test has given satisfactory results on over one hundred spinal fluids and compares favorably with the Lange's colloidal gold reaction.

In tabes dorsalis the test is less sensitive than the Lange's reaction.

The test does not call for special technical skill and for special glass-ware and triple distilled water. It can easily be performed in small laboratories or in the office.

#### REFERENCES

- <sup>1</sup>Takata, M, and Ari, K. A new colloidal liquor reaction and its practical results, Tokyo, 1926 (in German)
- <sup>2</sup>Blum, E. Ztschr f d ges Neurol u Psychiat, 1927, cx, 504
- <sup>3</sup>Jacobsthal, E, and Joel, M. Klin Wchnschr, 1927, vi, 1896
- <sup>4</sup>Hantsch, E. Psychiat Neurol Wchnschr, 1927, lxxx, 3
- <sup>5</sup>Knigge, F. Munchen med Wchnschr, 1926, lxxvii, 1836
- <sup>6</sup>Nicole, R. Ztschr f Klin Med, 1927, cxv, 193

## A CRITIQUE OF THE LIPASE PICTURE' METHOD''\*

By K. GEORGE FALK, HELEN MILLER NOYES AND KANEMATSU SUGIURA  
NEW YORK

IN A PAPER with the above title<sup>1</sup> Harry Beckman M.D., expresses disappointment with the method of determining and presenting certain ester hydrolyzing actions by us.

The disappointment of Beckman is stated to be based upon three sets of objections. Work on rat spleen extracts only is quoted.

1 Serum lipase is not taken into account in the method. No experimental evidence is presented by Beckman as to the amount of serum present in the material or of the relative lipase activities of the serum and tissue extracts. The criticism is based upon the finding of a serum lipase by Rona and Michaelis in 1911 and by Rona and Bien in 1914 and upon the different colors of ten splenic extracts prepared by Beckman. It may be added that in some unpublished experiments carried out in the Harriman Research Laboratory, it was found that the action on benzyl acetate of rat serum was between 50 and 75 per cent of the action of phenyl acetate while with the rat spleen extract it was less than 14 per cent. This in itself would show that the serum if present in the spleen exerted a negligible influence.

2 Whether the rats are killed by a crushing blow on the head or by ether inhalation is stated to show different results with the spleen extracts. The method of testing the enzyme actions of Falk, Noyes and Sugiura was not used by Beckman but a 'modification to meet experimental conditions' was developed. The extracts used by Beckman were very much more dilute than those used in the original method. Beckman in Table I of his paper gives ten results on extracts of spleens of individual rats on methyl butyrate. In the first place using N/100 NaOH gives numbers comparable in magnitude to those found in the original method where N/10 NaOH was used but the actions when translated into the latter terms ranged in the extreme cases from 0.04 to 0.55 cc. Nothing is said as to whether duplicate determinations were made. The extracts were stated to be 'neutralized' before incubation with the esters, but whether indicator was added to the solutions and was present throughout the tests and whether a definite  $P_H$  was attained is not stated. If the actions on methyl butyrate were the largest found obviously the extent of the ester hydrolyses in general was rather small upon which to base final conclusions. It may also be asked why results on rat spleen only were presented by Beckman and detailed results (in Table I and Fig. 2) on methyl butyrate alone to show the irregular actions, when it was specifically stated on page 203, line 2, of the paper by Falk, Noyes and

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Sugiura, in connection with the rat spleen results that "the methyl butyrate actions were somewhat irregular"

However, Beckman plots his experimental results on six esters (including benzyl benzoate which was not used in the original method), and then states \* "It will be seen that there is a certain characteristic 'picture' for all the animals in the group killed by a blow, but that very considerable quantitative differences exist between individuals of the group. In the group killed by ether the same type of 'picture' is again seen, but the quantitative differences between individuals are even greater than in the blow group." In view of the inaccuracies introduced by Beckman in his modification of the method, his conclusion agrees remarkably with the conclusions given in the original paper. There it was stated in the Discussion, page 211 "The results which have been presented show definite characteristic 'pictures' for the ester-hydrolyzing actions of the rat tumor and tissues. The relative actions of the various enzyme materials proved to be of the most significance in the interpretation of the results, but the absolute actions should also be considered." Also, on page 188 "In some cases the amounts of actions for the same concentration of a given tissue in different series differed considerably." Furthermore, a study of the curves given by Beckman in which the absolute actions, not the relative actions, were plotted, shows considerable variation in each set, and overlapping of the two sets. The two sets of curves, one obtained with rats killed by a blow, the other with rats killed by ether, are not distinct. Except for three results with one ester, methyl butyrate, for which the results are admittedly variable, it would not be possible to state definitely to which set a given curve belonged.

Evidently Beckman has contributed nothing, either constructive or destructive, in his experimental study.

3 The method is stated not to take into account the escape of esters from contact with the extract. The only experiment which Beckman gives in this connection (aside from indicating boiling points in a graph) is as follows "Exposing equal surfaces of the substances in calibrated tubes in the incubators for a given time will show the quite different rates at which they disappear." In the original method 0.2 to 0.5 cc of esters were present in each case with 15 cc of extract or mixture in a stoppered 60 cc Erlenmeyer flask. It is difficult to see how Beckman's experiment with pure esters applies to the method. He presents no evidence that esters evaporate either at different rates or at all under the conditions of the experiments.

#### CONCLUSION

The objections expressed in the "Critique" of Beckman are unfounded.

#### REFERENCES

- <sup>1</sup>Beckman, H. JOUR LAB AND CLIN MED, 1927, xii, 214  
<sup>2</sup>Falk, K. G., Noyes, H. M., and Sugiura, K. Jour Biol Chem, 1924, lix, 183

\*Italics were not used by Beckman

# CONTROL TESTS OF A HALDANE CHAMBER APPARATUS IN THE METABOLIC STUDY OF ADULT ALCOHOLIZED POULTRY\*

By THORNE M. CARPENTER PH.D., BOSTON, MASS

IN CONNECTION with a study begun in 1918 of the metabolism of poultry as affected by the inhalation of the vapor of ethyl alcohol, a number of gaseous exchange measurements were made upon several adult hens using the Haldane (1892) chamber method for animals. Occasionally results were obtained which were distinctly aberrant and it was thought advisable to study the difficulties of this method by running control tests with burning ethyl alcohol.

The general principle of the method is as follows. An airtight chamber containing the animal is ventilated by a current of air free from carbon dioxide and water vapor, the carbon dioxide and water vapor in the air leaving the chamber are retained by suitable absorbers which are weighed periodically. The chamber containing the animal is similarly weighed and the oxygen absorbed by the animal is calculated by subtracting the loss in weight of the chamber and contents from the sum of carbon dioxide and water vapor.

In the decade following its description by Haldane, the method was used by Pembrey and coworkers for mice (Pembrey 1894) rabbits (Pembrey and Gurber 1894, Mutch and Pembrey 1911 Kennaway and Pembrey 1912), eggs and chicks (Pembrey, Gordon and Warren, 1894), marmosets dormice and hedgehog (Pembrey, 1901 1903), and rats (Pembrey and Spriggs, 1904). During the last decade it has been more widely applied both biologically and geographically. Since Danoff (1919) used it in the study of the metabolism of rats it has been employed in the Institute of Physiology at Berne in an extensive series of studies with small animals. The method has been applied by others to the metabolism studies on rats (Fridericia 1913 Cramer and McCall, 1917, Nakayama, 1924, Reiss and Weiss, 1925 Harris 1925 Mitchell and Carman, 1926, and Lee 1927), rabbits (Marine and Lenhart 1920, Bertsch 1920 Isenschmid 1920, Dittler, 1922 Tale, 1922 and Okuma, 1926) dogs (Hédon, 1921, 1922, 1926), and chickens (Mitchell and Haines, 1927 a, 1927 b). Terroine and coworkers (Terroine and Trautman, 1926, 1927) have used it on a variety of small animals. Maime (1922) gave a detailed description of the apparatus as applied to infants rabbits, and cats, and Hédon (1926) described the apparatus as applied to dogs and discussed the magnitude of the errors theoretically possible. Boothby and Sandiford (1924) comment that 'as the oxygen is indirectly determined \* \* \* the respiratory quotient may be considerably disturbed by the fact that a large error may result from the indirect method of oxygen determination on account of the

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difficulty of accurately measuring the water vapor" Krogh (1916) stated that "the Haldane apparatus, which allows the determination both of carbon dioxide and oxygen is extremely accurate"

Many of the users of this method state that blanks have been run, tests for tightness made, and efficiency tests of the absorptive power of the water and carbon dioxide retainers have been conducted, but very few have published any numerical results. Some have taken the low respiratory quotients found with fasting animals as an indication that the method was correct. Haldane (1892) and Pembrey and Guiber (1894) reported quantitative results for the recovery of known amounts of water and carbon dioxide, Hauri (1919) determined the error in the water determination, and Isensee and Schmidt (1920) standardized his apparatus with known amounts of carbon dioxide developed by the addition of hydrochloric acid to calcium carbonate. It is recommended that investigators give at least a quantitative summary of blank tests and controls of efficiency of absorption.

A control of the Haldane chamber apparatus in experiments on animals was made by Fredericia (1913) in which he used a closed circuit system both as a Regnault-Reiset apparatus and as a Haldane apparatus. The oxygen absorption was determined from the contraction in volume of a gasometer connected to the circulating closed system, and the water vapor and carbon dioxide output were determined by the gains in weight of absorbers containing sulphuric acid and soda lime. At the end of each period the chamber was detached and weighed so that the oxygen absorption could be calculated indirectly as well as directly determined. Rats were used in 13 experiments from two and one-half to twenty-four hours in duration and the agreement between the two methods of oxygen determined averaged inside of 0.5 per cent, when the length of the experiment was more than three and one-half hours.

Cori and Cori (1926) have made a similar comparison on rats in 40 experiments, of the oxygen determined by means of the closed circuit with that obtained by the Haldane method, and report an average agreement of 1.4 per cent for three to four hour periods. The average difference in the respiratory quotients was 0.01. In a recent paper (Cori and Cori, 1928) they confirm their earlier results.

#### CONTROL TESTS WITH BURNING ETHYL ALCOHOL

*Apparatus and Technique*—The chamber used in the series reported here was made of sheet copper and was 37 cm long, 23 cm wide, and 28 cm high with a volume of about 25 liters. An opening at the top made of brass angle was 21 cm long and 16.5 cm wide. This is large enough to permit the entrance of a 2 to 3 kg fowl. A pane of plate glass was sealed in with physicists' wax to cover the opening. At one end of the bottom of the chamber two steel pivots were fastened on the outside which rested in sockets on a wooden frame. The other end of the bottom rested on two metal springs. At the top of the chamber above the end which rested on springs, a pointer of sheet metal was attached. With this arrangement, the movements of an animal inside of the chamber were registered upon the smoked paper on the moving drum.

of a kymograph. The amplitude of the record depended upon the length of the pointer and the sensitivity depended upon the tension of the springs on which one end of the chamber rested. The ventilating air entered at a corner in the lower part of the chamber wall and went out at the top at a diagonally opposite point. On the end where the ventilating air left a thermometer was inserted in a tubular enlargement in order to obtain the temperature of the air in the chamber.

*Absorber Systems*.—The ventilating air from the room or from out of doors passed to the chamber through a calcium chloride tower filled with soda lime and through two Drechsel gas washing bottles containing concentrated sulphuric acid. From the chamber it then passed through two Drechsel bottles containing sulphuric acid, a calcium chloride tower filled with soda lime, two Drechsel bottles containing sulphuric acid, a bottle containing a solution of barium hydroxide, a 10 liter Bahr gas meter and a small rotary blower. The purpose of the barium hydroxide container was to make sure that the carbon dioxide was completely absorbed from the incoming air from the chamber. If the second of each pair of water absorbers was weighed a control was maintained of the efficiency of the water absorption. So much trouble developed from time to time with cracks appearing in the wax seal of the opening of the chamber that it was found advisable to test the completeness of absence of leaks at the end of each period by putting suction on the chamber and absorber system equivalent to more than that obtained under running conditions. After the chamber was weighed it was connected to the absorber train following it and suction was applied to this system the incoming tube of the chamber being closed and the manometer placed at the exit of the last bottle of the absorber train. In most cases however the absorber train was tested while the chamber was being weighed and after weighing the chamber was tested alone. The leaks in the chamber were usually due to the contraction of the wax away from the metal. This procedure was recommended by Haldane (1892) and its use in our experiments with poultry has proved valuable in detecting leaks in the system which might have passed otherwise unnoticed. It is to be strongly recommended as an essential part of the routine with the Haldane chamber method.

*Alcohol Lamp and Technique of a Control Test*.—The lamp used was an Erlenmeyer flask or bottle of 100 cc capacity into which was inserted a rubber stopper with a wick (cotton string) running through glass tubing placed in a hole of the stopper. The bottle was partially filled with ethyl alcohol of known density, lighted and its rate of burning determined by weighing on a balance at intervals and adjustment of the rate (1 to 2 gm per hour) was made by raising or lowering the wick. The lighted lamp was then placed in the chamber and the latter was then connected to the ventilating air current which varied from 4 to 95 liters per minute in these experiments. The opening of the chamber was then sealed and the ventilation continued for about one half hour. The chamber was then closed off from the ventilating air current by screw pinch cocks on rubber tubing connected to the ends and was weighed as quickly as possible. A Sauter balance with a sensitive



ity of one centigram under a load of 5 kg was used for all the weighings. As soon as possible after the weighing the chamber was connected to the ventilating system and the air current started through a previously weighed set of absorbers for water vapor and carbon dioxide. Occasionally too much time was taken for weighing the chamber and the flame went out, but when care was taken to place beforehand on the balance pan the estimated weight of the chamber, little difficulty was experienced in this regard.

#### RESULTS OF ALCOHOL CHECK EXPERIMENTS

The results of 8 experiments are shown in Table I. In these experiments it was assumed that the losses in weight from the chamber represented the weights of alcohol which were burned in the individual periods. This assumption would mean that there was no change in weight of the chamber other than that due to the loss due to the burning of alcohol, i.e., no extra water was vaporized and the composition of the residual air remained unchanged. The periods are for the most part of thirty minutes' duration or more, but the weight of alcohol burned is of more significance than the length of the periods. The alcohol burned was usually over 1 gram, but in Experiment 8 it was 0.43 gram. In all experiments, except No. 7, there was a decrease in the amount of alcohol burned in each succeeding period. This is the usual experience with alcohol burning in a lamp such as was used in these experiments. The percentage of water recovery is on the whole higher than theory, which would indicate that the loss in weight of the chamber included some vaporized water other than that which came from the alcohol. The weights of water were for the most part over 1 gram, with the exception of periods in Experiments 2 and 8. The carbon dioxide on the whole averages slightly lower than theory, indicating that there was less alcohol burned than computed from the loss in weight of the chamber. The weights of carbon dioxide absorbed in the individual periods are somewhat higher than those for the water, for the most part over 2 grams. The oxygen recovery on the whole is slightly better than that for carbon dioxide and the quantities in the various periods are slightly higher than those for carbon dioxide. The quantities of water vaporized, carbon dioxide produced, and oxygen used, are for the most part not much larger than those involved in the metabolism of adult hens. For 132 periods in 25 experiments with hens in which the duration of the periods averaged fifty-three minutes, the values were: water vaporized, 1.75 grams, carbon dioxide eliminated, 1.79 grams, and oxygen absorbed, 1.61 grams. These alcohol check tests form a suitable basis for estimating the accuracy of results in studies with adult poultry.

The most important calculation in the table is the respiratory quotient in the different experiments. Experiment 5 was the best in individual periods but for the most part the average of each experiment comes within 0.02 of the theoretical respiratory quotient for alcohol. It is evident that with extreme carefulness, respiratory quotients may be obtained for individual periods with this method, which are very satisfactory as shown by Experiment 5 and the two periods of Experiment 2. As the respiratory quotients for the experiments as a whole are satisfactory, the indication is that the

TABLE I

RESULTS OF ALCOHOL CHECK EXPERIMENTS WITH A HALDANE CHAMBER RESPIRATION APPARATUS

EXPERIMENT NUMBER AND PER CENT OF ALCOHOL	DURATION OF PERIOD	LOSS FROM CHAMBER (ALCOHOL)	WATER		CARBON DIOXIDE		OXYGEN		RES PIRATORY QUOTIENT
			FOUND	PER CENT OF THEORY	FOUND	PER CENT OF THEORY	FOUND	PER CENT OF THEORY	
	HR. MIN.	GM.	GM.		GM.		GM.		
1	46	0.98	1.22	108.0	1.77	103.5	2.01	107.5	0.640
91.3 per cent	46	0.97	1.12	100.0	1.57	92.9	1.72	93.0	0.664
	46	0.97	1.16	103.6	1.60	94.7	1.79	96.8	0.650
	46	0.83	1.03	107.3	1.45	100.0	1.65	104.4	0.639
Total	3 4	3.75	4.53	104.1	6.59	97.7	7.17	100.4	0.647
2	45	0.71	0.83	101.2	1.30	104.8	1.42	105.2	0.666
91.3 per cent	45	0.56	0.62	95.4	1.01	103.1	1.07	100.0	0.686
Total	1 30	1.27	1.45	98.6	2.31	104.1	2.49	102.9	0.678
3	60	1.82	2.16	102.4	2.92	91.8	3.26	94.2	0.651
91.3 per cent	62	1.70	1.94	98.5	2.85	96.0	3.09	95.4	0.671
	60	1.52	1.92	109.1	2.80	105.7	3.20	110.7	0.637
	60	1.51	1.87	106.9	2.77	105.3	3.13	109.1	0.644
	60	1.40	1.58	97.5	2.35	96.3	2.53	95.1	0.675
Total	5 2	7.95	9.47	102.8	13.69	98.7	15.21	100.5	0.654
4	62	1.31	1.45	90.7	2.05	96.7	2.19	94.8	0.681
84.5 per cent	60	1.17	1.37	102.2	1.78	94.2	1.98	96.1	0.654
	60	1.10	1.33	105.6	1.62	91.0	1.85	95.4	0.637
	60	1.02	1.27	108.5	1.56	94.5	1.81	100.0	0.627
Total	4 2	4.60	5.42	102.7	7.01	94.3	7.83	96.7	0.651
5	60	2.01	2.35	101.7	3.06	94.2	3.40	96.0	0.655
84.5 per cent	60	1.67	1.97	102.6	2.73	101.1	3.03	103.1	0.655
	60	1.50	1.78	103.5	2.70	105.4	2.83	107.2	0.655
	60	1.54	1.78	100.6	2.53	101.0	2.77	102.2	0.664
	60	1.49	1.71	100.0	2.34	97.1	2.56	97.3	0.665
Total	5 0	8.21	9.59	101.8	13.21	99.6	14.59	100.9	0.659
6	30	1.24	1.48	104.2	1.89	94.5	2.13	97.7	0.645
84.5 per cent	30	1.07	1.22	99.2	1.73	100.0	1.88	99.5	0.670
Total	1 0	2.31	2.70	101.9	3.62	97.1	4.01	98.5	0.655
7	63	1.18	1.43	105.9	2.01	105.2	2.26	108.7	0.647
84.5 per cent	64	1.00	1.51	101.3	2.39	99.5	2.30	100.4	0.661
	64	1.28	1.45	98.6	2.03	98.1	2.20	97.3	0.672
	64	1.25	1.41	98.6	1.94	96.0	2.10	95.5	0.672
Total	4 15	5.01	5.80	100.9	8.07	99.8	8.86	100.3	0.665
8	45	1.18	1.32	97.8	1.78	93.2	1.92	92.3	0.674
84.5 per cent	45	1.19	1.36	100.0	1.91	99.5	2.08	99.0	0.668
	25	0.43	0.46	93.9	0.63	98.6	0.71	93.4	0.697
Total	1 55	2.80	3.14	97.8	4.37	96.7	4.71	95.5	0.673
Average of periods	--	1.24	1.45	101.8	2.02	98.4	2.24	99.6	0.659
Average de- viation of periods	--	0.27	0.32	3.2	0.47	3.8	0.52	4.4	0.013
Mean devia- tion of periods	--	0.36	0.43	4.0	0.59	4.5	0.66	5.3	0.016

difficulty of obtaining theoretical results for carbon dioxide and water in the individual periods is not due to the variation in the quantities themselves but due to the difficulty of measuring accurately the amount of alcohol burned. If there were appreciable errors in any one of the three factors measured the result would be an error in the fourth factor which is calculated, namely oxygen, and an error in the ratio between carbon dioxide and oxygen that is the respiratory quotient.

The average deviation and the mean deviation of the periods are given at the bottom of the table. For the quantities measured these two constants show the degree of variability of the amounts of the weights of losses of the chamber, water, and carbon dioxide. On the other hand the constants for the percentages show the variability in the accuracy with which the observations were made. In general the average deviation of the percentages is between 3 and 4.5, while the mean deviation of the percentages is between 4 and 5.5. Out of a total of 29 periods, sixteen of the periods for the water, fifteen of the periods for carbon dioxide, nineteen of the periods for oxygen, and twenty of the periods for the respiratory quotient are within the mean deviation. As the mean deviation is less percentagewise for the respiratory quotient than for any of the other three factors, it is easier to obtain quotients in alcohol checks than it is to measure water, carbon dioxide, and oxygen. The reason for this is that the respiratory quotient depends upon the accuracy of the weighings of the individual parts, while the percentage recovery of oxygen, carbon dioxide, and water depends upon whether the loss in weight of the chamber represents solely the alcohol burned.

As there was no source of carbon dioxide other than the burning alcohol, and the completeness of absorption was controlled by the barium hydroxide solution, the weight of carbon dioxide obtained was used as the basis of calculating the amount of alcohol burned and the percentages of recovery for oxygen and water were computed. The average percentages for water and oxygen were 101.5 and 101.1 respectively, so that on the whole the results are not much changed for the average of the individual experiments on the assumption that the carbon dioxide was an indication of the alcohol burned. The calculation of the average deviation and the mean deviation for the individual periods when carbon dioxide is assumed as the measurement of the alcohol burned gives 2.1 and 2.6 per cent respectively for the oxygen which is of the same order as those for the respiratory quotient.

These experiments show that the Haldane chamber respiration apparatus will give reliable respiratory quotients with adult poultry when the periods are at least two hours in length and a balance sensitive to 0.01 gram with a load of 5 kilograms is used. For shorter periods it is necessary to have a more sensitive balance, although there are a number of periods (of one hour duration) in this series which are satisfactory so far as respiratory quotient is concerned. The greatest difficulty is in weighing the chamber, particularly with a live animal in it. Care should be taken to maintain a uniform rate of ventilation so as to have the composition of the residual air as nearly the same at the end of each period as possible, and to perform the weighing in the same length of time at each period, as unequal duration of weighings results in unequal changes in temperature of the chamber, which affect the weight of the chamber. When these precautions are observed and a suitable balance is used, the method should give trustworthy results in the measurement of respiratory exchange of small animals.

All measurements are made with one instrument (the balance), and therefore, the factors measured should all have the same degree of accuracy. The equipment required can be assembled easily and with the exception of

the chamber can be readily purchased. The technique is not complicated and therefore, can be acquired quickly. The method has also the great advantage that several sets of apparatus can be used and only one balance is required. In view of the simplicity of the method both in theory and in equipment required it is surprising that it has had so little application until recent years.

## SUMMARY

Control tests with ethyl alcohol were made with a Haldane chamber respiration apparatus suitable for adult poultry. The average percentage recovery for water, carbon dioxide and oxygen in 8 experiments of one hour to five hours in duration was 101.8, 98.4 and 99.6 respectively. The average respiratory quotient was 0.659. The mean deviation from average in 29 periods was for water 4.0, carbon dioxide 4.5, and oxygen 5.3 per cent. The mean deviation of the respiratory quotient from 0.659 was 0.016.

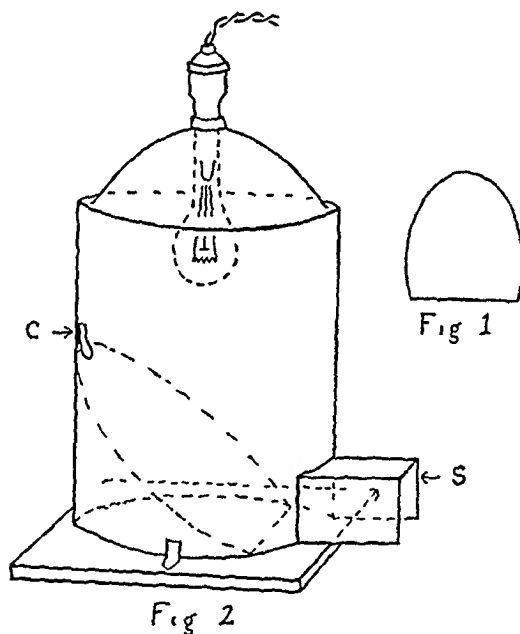
## REFERENCES

- Bertsch, H. *Biochem. Ztschr.* 1920 cxi 37.  
 Boothby, W. and Sandiford, I. *Physiol. Rev.* 1924 iv, 75.  
 Cori, C. F., and Cori, G. T. *Jour. Biol. Chem.*, 1926, lxx, 560. *Jour. Biol. Chem.*, 1928, lxxvi, 759.  
 Cramer, W., and McCall, R. *Quart. Jour. Exper. Physiol.* 1917 xi 61.  
 Dinoff, A. *Biochem. Ztschr.* 1919 xciii 44.  
 Dittler, R. *Ztschr. f. Biol.* 1922 lxxvii 143.  
 Frederica, J. S. *Biochem. Ztschr.* 1913 lix 9.  
 Haldane, J. S. *Jour. Physiol.* 1892 xiii 419.  
 Harris, D. T. *Rev. Soc. Proc.* 1922, Ser. B xxiiv 171.  
 Hédon, L. Thèse Montpellier 1921. *Arch. internat. de physiol.* 1922, xvi, 4. *Arch. internat. de physiol.* 1926 xvi 3-9.  
 Isenschmid, *Arch. f. exper. Path. u. Pharmacol.* 1920 lxxxv 271.  
 Kennaway, F. L., and Pembrey, M. S. *Jour. Physiol.* 1912 xlv 8.  
 Krogh, A. *The Respiratory Exchange of Animals and Man* 33. Longmans, Green & Co., New York 1916.  
 Lee, M. O. *Am. Jour. Physiol.* 1927 lxxxi 402.  
 Marine, D. *Jour. Metab. Res.* 1922 ii 29.  
 Marine, D. and Lenhart, C. H. *Am. Jour. Physiol.* 1920 li 348.  
 Mitchell, H. H. and Cirman, C. G. *Am. Jour. Physiol.* 1926 lxxvi 387.  
 Mitchell, H. H. and Haines, W. T. *Can. Jour. Agric. Res.*, 1927, xxix, 549. *Can. Jour. Agric. Res.*, 1927, xxix, 92.  
 Mutch, N. and Pembrey, M. S. *Jour. Physiol.* 1911 xliii 109.  
 Nakajima, K. *Biochem. Ztschr.* 1924 cxlviii 98.  
 Okumura, T. *Biochem. Ztschr.* 1926 cxlxxv 32.  
 Pembrey, M. S. *Jour. Physiol.* 1894, xi 401. *Jour. Physiol.* 1895 xviii 360, *Jour. Physiol.* 1901, xxvii 66. *Jour. Physiol.*, 1903 xxx 19.  
 Pembrey, M. S., Gordon, M. H. and Warren, R. *Jour. Physiol.* 1894 xvii 331.  
 Pembrey, M. S. and Gürber, A. *Jour. Physiol.* 1894 xi 449.  
 Pembrey, M. S. and Spriggs, E. I. *Jour. Physiol.* 1904 xxx 320.  
 Reiss, M. and Weiss, R. *Klin. Wchenschr.* 1922, iv 2207.  
 Také, N. M. *Jour. Infect. Dis.* 1923 xxvii 1-8.  
 Terroine, E. F., Trautman, S. and Schneider, J. *Ann. de Physiol.* 1926 ii, 468.  
 Terroine, E. F. and Trautman, S. *Ann. de Physiol.* 1927 iii 422.

## A LAMP FOR THE COLORIMETER OR MICROSCOPE\*

BY H. A. DAVENPORT, M.D., CHICAGO

ARTIFICIAL light suitable for either colorimetric or microscopic work should be a good imitation of daylight, and be supplied in a diffuse beam which is large enough to cover the reflector or mirror of the instrument used. Such a source of light is produced by the lamp described here.



The body of the lamp is made from a standard two gallon gasoline or kerosene can. The spout is removed and the hole covered by soldering on a metal patch. A standard brass-shell electric light socket is soldered into the collar which is normally covered by the filler cap of the can. The bottom of the can is cut out, and a rectangular hole about  $2\frac{1}{2}$  inches high by 4 inches wide cut in the side at the lower edge. A metal shield (s, Fig 2) shaped like a broad inverted U is soldered around the edge of the rectangular hole. This shield should project 3 or 4 inches from the side of the can. A reflector, semi-oval in shape, is made from any thin material which has a plane, smooth surface. If metal is used, care should be taken that it is flat. The reflector should be fitted at an angle of  $45^\circ$  and a small metal chip (c, Fig 2) soldered to the inside wall of the can opposite the opening and at the height necessary to hold the top of the reflector. Bottom supports for the reflector are not nec-

\*From the Northwestern University Medical School, Chicago.  
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essary if it fits well. Two small brass angle irons with screw holes in their ends are soldered to the sides of the bottom for attaching it to the base.

The inside of the can and the reflector are painted (two coats) with aluminum paint. The outside will probably tire the eyes least if painted dull black.

A base is made of a piece of board 10 inches square. It should be just thick enough to bring the opening of the lamp level with the reflector of the microscope or colorimeter, or varying heights may be obtained by "shimming up" the lamp with additional squares of thick cardboard.

The order of assembly is as follows. Insert a 100 watt blue glass (day light) bulb into the socket. Adjust the reflector so that the beam is thrown straight out the "tunnel" made by the shield, and screw the can to the base.

Fig. 1 illustrates the shape of the reflector. Fig. 2 is a skeleton diagram of the completed apparatus showing the placement of the reflector.

The device can be constructed by a tinner in an hour or two at a cost of a few dollars, and will prove to be a handy substitute for daylight in the laboratory. The color value given for blue will be found much superior to those given by an ordinary electric light. Used for microscopic work it will be found superior to the substage lamp.

Points to be emphasized are: use aluminum paint (not white), use a blue glass, nitrogen filled bulb, and set the reflector at such an angle that both sides of a colorimeter field are illuminated equally.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## LABORATORY TECHNIC

**TISSUE STAIN** A Modified Methylene Azure B Stain for Sections of Human Hematopoietic Organs, Richter, M. N. Arch Path and Lab Med, 1927, 11, 1, 773

- 1 Fix (A) pieces not more than 2 mm thick by Ellemann's method  
Twenty four hours in freshly prepared mixture of Zenker (without acetic acid) 9 parts  
Neutral formalin (40 per cent formaldehyde) 1 part
  - 2 Wash in running water, from twelve to twenty four hours
  - 3 Embed in eeloidin (B) (or paraffin)
  - 4 Attach sections to slide by albumin and remove eeloidin with absolute alcohol and ether (b) (or paraffin with xylene)
  - 5 Absolute alcohol, one minute
  - 6 Ninety five per cent alcohol containing tincture of iodine, fifteen minutes
  - 7 Eighty per cent alcohol until iodine is removed
  - 8 Water several changes to remove alcohol
  - 9 Stain fifteen minutes, while staining (C) in freshly prepared mixture of  
Erythrosin, 1 per cent aqueous 50 cc  
Neutral formalin 0.25 cc  
Eosin or phloxine may be substituted for erythrosin  
rosin
  - 10 Rinse in distilled water
  - 11 Stain from one to five minutes in  
Methylene azure B bromide 0.1  
Methylene blue USP medicinal 0.1  
Distilled water, or preferably buffer solution, pH 7.5 100.0
- The buffer is made by adding 15 cc of a fifteenth molecular solution of primary potassium phosphate (9.078 gm to 1 liter of distilled water) to 85 cc, of secondary sodium phosphate solution (11.876 gm to 1 liter)
- 12 Flood from dropping bottle with several changes of absolute ethyl alcohol, until stain ceases to come out (ten seconds at least) Avoid water and lower alcohols
  - 13 Differentiate in  
Clove oil 3 parts  
Absolute ethyl alcohol 1 part  
Colophonium, sat. alc. solution a few drops
- Control the differentiation under the microscope
- 14 Place for thirty seconds in (D)  
Xylene 9 parts  
Amyl alcohol 1 part
- The differentiating fluid must be thoroughly removed
- 15 Pure amyl alcohol, until ready to mount
  - 16 Mount in euparal, preferably the green variety

### Results

The nuclei are a deep blue black and the chromatin is sharply stained. The nucleoli are distinct. The cytoplasm is various shades of lavender, red or blue, depending on cell types. The eosinophil granules are red, the neutrophil granules slightly

darker red, and the basophil granules blue. The centrosomes are a bright red. The spindle fibers are usually red and sometimes are purple. The fibrin is red or purple, depending on the degree of decolorization. The bacteria are blue.

**LIVER FUNCTION** *Rose Bengal Test for Liver Function* Epstein N N Delprat G D and Kerr W J *Jour Am Med Assoc* 1927, LXXXIII 1619

The authors report the results of their continued study of the test described by them in 1923 the present report summarizing the findings in more than 500 cases.

The modified technique follows:

**Injection.** A vein in the cubital fossa is selected and 10 cc of a 1 per cent solution of rose bengal in physiologic sodium chloride solution is injected and the needle washed through slowly with from 5 to 10 cc of salt solution which is held ready in a fresh syringe. The needle is left in the vein and at exactly two minutes after the injection of the dye a sample of blood (10 cc) is withdrawn from the needle into a clean syringe and discharged into a centrifuge tube containing a few crystals of potassium oxalate. The tube is carefully inverted two or three times. The needle is again washed by slowly injecting from 5 to 10 cc of the salt solution which maneuver prevents the clotting of blood in the needle. At eight and sixteen minute respectively from the time of injection, samples of blood are withdrawn and collected in an identical manner. The needle is then withdrawn from the vein in the arm. It is well to advise the patient that the appearance of the dye in the veins will impart to them a distinct red color, since the unexpected appearance of the red tints may cause considerable alarm.

As soon as possible after collection the blood samples are centrifuged at a speed of 2000 revolutions per minute for thirty minutes.

Ten cc record syringes and number 16 gauge recurved needles are convenient. Rough handling of the blood samples is apt to cause some hemolysis of the red cells and to interfere with the colorimetric readings.

**Colorimetric Analysis.** Five cubic centimeters of the plasma of each of the samples is separately mixed with 10 cc of physiologic sodium chloride solution. The diluted plasma of the two minute sample is placed in the wedge of the Hellige colorimeter. This is used as the standard against which the eight and sixteen minute diluted samples are read.

Since the two minute sample represents the highest concentration of the dye in any individual, following the injection of a fixed amount of the dye into the circulation it may be regarded as 100 per cent retention and the colorimetric readings obtained by comparing the eight minute and sixteen minute specimens with it represent direct percentages of dye retained. Before making colorimetric readings it is necessary to standardize the Hellige colorimeter used and to correct for any inaccuracies present. The colorimeter is standardized by comparing one sample of plasma against itself the reading should be 100.

The colorimetric readings thus obtained indicate the ratio between the first or control sample and the subsequent sample. This obviously indicates the speed with which the dye leaves the circulation.

While the two minute sample is regarded as representing the highest concentration of the dye in an individual at any one time it is probable that some of the dye is removed from the circulation before the collection of the two minute sample but this is not of great practical importance if care is taken to withdraw the blood at exactly two minutes. Furthermore we are not so much concerned with the actual quantity of dye in the circulation as with the ratio of the percentage of dye in circulation at two distinct intervals of time which in reality is its rate of disappearance. For this reason it is not essential that the solution of dye injected be exactly a 1 per cent solution nor that the dose be exactly 10 cc. Although considerable latitude may be taken in regard to the amount of dye injected it would be advisable to inject approximately 100 mg in the average sized adult. The dose may be varied according to the size of the patient for some endeavor should be made to keep the initial concentration of the dye approx



imately the same. The dosage has varied from 3 cc of a 1 per cent solution in children to 15 cc in large adults. In normal individuals, the eight minute sample is usually 50%, varying from 40 to 60 per cent, and the sixteen minute sample varying from 23 to 30 per cent. These percentages represent the amount of dye retained in the circulation.

The results may be thus summarized:

Normal persons absorb rose bengal through the liver from the blood stream very rapidly so that at the end of sixteen minutes only a trace remains in the blood plasma, that is, rarely more than 25 per cent of the amount injected.

In chronic cholecystitis the elimination is usually within normal limits, and the test is not an aid in the diagnosis of the condition.

In cases of obstructive jaundice, catarrhal jaundice and arsphenamine icterus, the delay in the elimination of the dye is always very definite, being greatest in obstructive jaundice. The test is a valuable aid in detecting these conditions and in following their clinical course. Before the jaundice begins to clear clinically the improvement in liver activity can be demonstrated by its increased ability to eliminate rose bengal. Following operations for the relief of obstruction of the common duct, the liver function improves markedly. The test may be used as an aid in determining the patency of a cholecystoduodenostomy, as the dye can be readily identified in the duodenal contents. It has proved very valuable in following the course of an arsphenamine hepatitis.

In cirrhosis of the liver, the impairment of liver function is in direct relation to the amount of scarring in the liver and to the encroachment on the functional reserve of the liver. An advanced case of cirrhosis always shows a marked delay in the excretion of rose bengal.

The test is of particular value in the differential diagnosis when ascites is the outstanding symptom. In cardiac failure, in tuberculous peritonitis and in carcinomatosis of the peritoneum, little change from the normal liver function has been noted, while on the other hand, when cirrhosis of the liver is present, there is a very marked impairment of liver function.

In metastatic malignancy of the liver, the conditions are variable and seem to depend on the amount of liver tissue which can function. Discrete scattered metastatic nodules in the liver do not interfere with its ability to eliminate the dye, but diffuse replacement of the liver tissue by carcinoma very definitely causes a retention of the dye in the blood stream.

Chronic passive congestion of the liver does not seem to prevent the absorption of rose bengal, and the curves are within normal limits.

Observations in patients in the various stages of syphilis have proved interesting, especially in those with a very florid type of early generalized infection. In a small percentage of these a definite delay in the excretion of the dye has been noted, and probably indicates the presence of an acute syphilitic hepatitis in spite of the clinical silence of the liver. Long continued treatment with arsphenamine and mercury does not disturb the liver activity. Arsphenamine dermatitis unaccompanied by icterus shows elimination of rose bengal within normal limits.

Acute infections of the liver cause a marked impairment of liver function.

Nephritic toxemias of pregnancy do not cause delay in the elimination of rose bengal from the blood stream.

Continued application of the test confirms previous observations as to its value as an aid in diagnosis and in estimating the prognosis of certain diseases of the liver.

#### **NERVE TISSUE STAIN** Stain Technique for Nissl's Granules Following Alcohol Formalin Fixation, Bean, R. J. Stain Technology, 1927, 11, 56

1 Nerve tissue is fixed 24 hours in a 5 per cent solution of strong formalin in commercial alcohol.

2 If dehydration is perfect, either chloroform or xylol may be used as a clearing agent.

3 A slow method of paraffin infiltration is advisable.

4 Sections should be cut 10  $\mu$  microns in thickness

5 Coplin staining jars should be annealed by placing them on a rack in a pan of cold water, bringing the water to the boiling point and allowing the jars to stand in boiling water for twenty minutes

6 One per cent aqueous solutions of either methylene blue or Grubler's Neutral Roth are used as specific stains for Nissl's granules

7 These stains are heated to boiling in a beaker, the slides are placed in the Coplin jars which are partially submerged in boiling water and the hot stain poured into the jars. The flame beneath the water bath is turned down and the slides left for 20 minutes

8 The excess of primary stain is washed off in 25 per cent and 50 per cent alcohol and the slides passed rapidly through the alcohol series to absolute alcohol, and finally to xylol

9 When counterstaining is desired nigrasin in 1 per cent aqueous solution, methyl orange, saturated solution in 50 per cent alcohol, or a 0.5 per cent solution of eosin in 50 per cent alcohol are recommended. These stains are used cold, and the slides are merely dipped in them after the excess of primary stain has been washed out in 25 per cent and 50 per cent alcohol

10 If a cold primary stain is desired a saturated solution of thionin in distilled water, acidified with 1 per cent carbolic acid will prove specific for the Nissl substance. Sections should be stained 5-10 minutes in thionin, then passed rapidly through to absolute alcohol, and xylol. The same counterstains may be used as in the hot method

11 Sections prepared by the hot method show little tendency to fade after ten years' use

12 Excepting neutral red, all the stains used in this technique are carried by the National Aniline and Chemical Company and are satisfactory. Coleman and Bell natural red may be substituted for Grubler's Neutral Roth with good results

#### SPIROCHETA PALLIDA A Modified Stain for Cerqueira's A. Brazil Med, 1927, xli, 258

##### Stain

Tannin C P	5 gm
Glacial acetic acid	4 cc
90 per cent alcohol	50 cc
Distilled water	50 cc

Chanera serum is smeared and fixed in 90 per cent alcohol 1 minute and without drying covered with the stain. The slide is heated until a little vapor is given off, washed in running water and covered with Ziehl-Neelsen carbolfuchsin and steamed for 10-20 seconds. Wash.

Treponema are pinkish violet

#### BLOOD GROUPING A New Method of Direct Matching Clemens J. Zentr. f. Chirurg 1926, lxxi, 3032

The following rapid method is proposed for compatibility tests

One or more drops of the recipient's blood are placed upon a slide and an equal volume of chloroform added the mixture being spread to twice the size of the original drop. The chloroform hemolyzes the blood and produces a clear red serum. If necessary more chloroform may be added and poured off. The procedure thickens the blood somewhat.

A drop of the donor's cells is then added to the hemolyzed serum and the mixture observed for agglutination.

The procedure is repeated testing the recipient's cells against the donor's hemolyzed serum.

**PANCREATIC ENZYMES** Estimation of, Martin, L Arch Int Med, 1927, *xxviii*, No 3, p 343

Determinations are made preferably upon B bile or, if this is turbid, upon clear A bile

After thorough centrifugalization 1 cc is placed in 19 cc of buffer solution  $P_{H\ 7.7}$  and 1 cc of the 1:20 mixture in 29 cc of the same buffer. This makes a 1:600 dilution. These two dilutions are sufficient.

The buffer solutions are prepared as follows:

An M/15 solution of primary potassium phosphate which contains 9.080 gm  $KH_2PO_4$  in 1 liter of solution.

An M/15 solution of secondary sodium phosphate which contains 11.876 gm  $Na_2HPO_4 \cdot 2H_2O$  in 1 liter of solution.

Nine cubic centimeters of the secondary are added to 1 cc of the primary to make  $P_{H\ 7.731}$ .

Into three Folin and Wu blood sugar tubes are placed respectively, 1 cc of the 1:20 dilution measured from an accurate 1 cc pipette, 0.1 cc from a 0.1 cc pipette, of the 1:20 dilution with 0.9 cc, standard buffer, 1 cc of the 1:600 dilution. To each tube is added 1 cc of 1 per cent starch solution made up by boiling as directed. This is incubated for thirty minutes in a water bath, 35° to 40° C and 2 cc alkaline copper sulphate of Folin and Wu added and carried out as directed by them for blood sugar determination. In normal cases the digestion in tubes 1 and 2 will be practically complete, certainly in 1. Tube 3 will be the only one necessary to read. If this reduction is low, it will be safe to take the 1:200 tube as representing the true pancreatic activity. This has been found to be the case as shown in the foregoing determinations in a series of five tubes with progressively smaller amount of each binary fraction in 1:600 dilution have been conducted. The milligrams of dextrose produced from one cc of starch are then calculated.

To arrive at a unit of comparison this simply necessitated the multiplication of the amount found in tube 3 by 100, in tube 2 by 33.3 and in tube 1 with a dilution of 1:20 by 33. For instance, in tube 3 the colorimetric reading of  $\frac{\text{Standard}}{\text{Unknown}} \times \text{amount of glucose in standard} \times 100 = \text{mg of glucose}$ , or to give an example using standard 2 of glucose working standard,  $20/15 \times 0.4 \text{ mg} \times 100 = \text{mg}$ . This represents 100 times the amount of glucose produced from one cc—1 per cent starch—by the action of 1:600 dilution of pancreatic enzyme as obtained by duodenal drainage.

**MILK** Can B. Coli be Used as an Index of the Proper Pasteurization of Milk? Swenarton, J. C. Jour Bact., 1927, *xiii*, No 6, p 416

Sixteen pasteurizing plants were studied with respect to the B. coli contents of the pasteurized milk.

The B. coli content of the pasteurized milk from the different plants was found to vary considerably.

The control charts from the plants whose milk was high in B. coli show improper heating or irregularity of procedure. There is a definite correlation between B. coli content and procedure as indicated by the control charts.

A test for B. coli in pasteurized milk can be used to good advantage in checking up on plant performance.

A standard is proposed for the maximum B. coli content of a properly pasteurized milk as follows:

The standard portion of milk shall be 0.1 cc. The standard sample shall consist of five standard portions of 0.1 cc each.

Of all the standard 0.1 cc portions examined, not more than 20 per cent shall show the presence of organisms of the B. coli group.

Occasionally three or more of the five (equal 0.1 cc) portions constituting a single standard sample may show the presence of B coli. This shall not be allowable if it occurs in more than (a) 10 per cent of the standard samples when ten or more samples have been examined or in (b) one standard sample when less than ten samples have been examined.

**ALCOHOL IN TISSUES** The Quantitative Determination of Alcohol in Human Tissues  
Gettler A. O. and Tiber A. Arch. Path. and Lab. Med. 1927, III, 75

The tissue as soon as it is removed from the body should be placed in a tightly closed jar and placed in a refrigerator. When needed 500 gm are weighed out quickly ground up and placed in a 2-liter flask. To this are added 100 cc of water, 5 cc of a saturated solution of tartaric acid and 1 cc of white mineral oil. This mixture is now distilled with steam. A long well cooled condenser should be used and the distillation should be continued until exactly 500 cc has been collected. The distillate is well mixed and used in the following procedure.

Twenty gram of potassium bichromate and 40 cc of concentrated sulphuric acid are placed in a 500 cc distilling flask. Three hundred cubic centimeters of the distillate obtained above are now added and the content mixed well. The flask is then connected to a long well cooled condenser by means of a Hopkins distilling head and the distillation started. The heat must be so regulated that it will take from forty five to fifty minutes to collect exactly 250 cc of distillate. After thoroughly mixing, 50 cc of this distillate is titrated with twentieth normal sodium hydroxide solution using phenolphthalein as an indicator. From this titration figure the amount of alcohol present in 1 kilogram of brain tissue can be easily calculated as follows: (cc twentieth normal alkali - 112)  $\times$  71.58 = mg  $C_2H_5OH$  per kilogram of tissue. The figure (- 112) holds only for brain tissue. For other tissue one must determine experimentally the corresponding blank.

**INTOXICATION** The Alcoholic Content of the Human Brain Gettler A. O. and Tiber A.  
Arch. Path. and Lab. Med. 1927 III, 114

For the qualitative and quantitative determination of alcohol the brain is of first importance.

The normal alcoholic content of human brain material is less than 0.0025 per cent.

The alcoholic content of the brain in persons who have partaken of alcoholic beverage ranges between 0.001 and 0.1 per cent.

All patients having an alcoholic content below 0.1 per cent (designated as 'one plus') show no abnormal physiologic effects.

Patients with an alcoholic content above 0.1 per cent and up to 0.25 per cent (designated as 'two plus') show some physiologic disturbance as evidenced by increased aggressiveness, and more or less loss of sense of care. None of these patients, however, shows unbalanced equilibrium which is commonly called intoxication.

When the alcoholic content rises above 0.25 per cent and up to 0.4 per cent (designated as 'three plus') and still higher up to 0.6 per cent ('four plus'), the equilibrium of the person becomes unbalanced—a condition generally known as intoxication.

The degree to which any person is affected does not depend on the quantity of alcohol consumed, but on the amount of alcohol present in the brain at the time.

**AMEBA** A Simplified Method for the Cultivation of *Endameba Histolytica* Craig C. F.  
Am. Jour. Trop. Med. Sept. 1926 VI, No. 5 p. 333

A medium consisting of Locke solution containing one part to seven of inactivated human, horse or rabbit blood serum has been found very efficient in the cultivation of *Endameba histolytica*. The best results were obtained when inactivated human blood serum was used.

**PANCREATIC ENZYMES** Estimation of, Martin, L Arch Int Med, 1927, *xxxix*, No 3, p 343

Determinations are made preferably upon B bile or, if this is turbid, upon clear A bile

After thorough centrifugalization, 1 cc is placed in 19 cc of buffer solution P<sub>11</sub> 77 and 1 cc of the 1:20 mixture in 29 cc of the same buffer. This makes a 1:600 dilution. These two dilutions are sufficient.

The buffer solutions are prepared as follows:

An M/15 solution of primary potassium phosphate which contains 9.080 gm KH<sub>2</sub>PO<sub>4</sub> in 1 liter of solution.

An M/15 solution of secondary sodium phosphate which contains 11.876 gm NaHPO<sub>4</sub> · 2H<sub>2</sub>O in 1 liter of solution.

Nine cubic centimeters of the secondary are added to 1 cc of the primary to make P<sub>11</sub> 7731.

Into three Folin and Wu blood sugar tubes are placed respectively, 1 cc of the 1:20 dilution measured from an accurate 1 cc pipette, 0.1 cc from a 0.1 cc pipette, of the 1:20 dilution with 0.9 cc, standard buffer, 1 cc of the 1:600 dilution. To each tube is added 1 cc of 1 per cent starch solution made up by boiling as directed. This is incubated for thirty minutes in a water bath, 38° to 40° C, and 2 cc alkaline copper sulphate of Folin and Wu added and carried out as directed by them for blood sugar determination. In normal cases the digestion in tubes 1 and 2 will be practically complete, certainly in 1. Tube 3 will be the only one necessary to read. If this reduction is low, it will be safe to take the 1:200 tube as representing the true pancreatic activity. This has been found to be the case as shown in the foregoing determinations in a series of five tubes with progressively smaller amount of each binary fraction in 1:600 dilution have been conducted. The milligrams of dextrose produced from one cc of starch are then calculated.

To arrive at a unit of comparison this simply necessitated the multiplication of the amount found in tube 3 by 100, in tube 2 by 33.3 and in tube 1 with a dilution of 1:20 by 33. For instance, in tube 3 the colorimetric reading of  $\frac{\text{Standard}}{\text{Unknown}} \times \text{amount of glucose in standard} \times 100 = \text{mg of glucose}$ , or to give an example using standard 2 of glucose working standard,  $20/15 \times 0.4 \text{ mg} \times 100 = \text{mg}$ . This represents 100 times the amount of glucose produced from one cc—1 per cent starch—by the action of 1:600 dilution of pancreatic enzyme as obtained by duodenal drainage.

**MILK** Can B. Coli be Used as an Index of the Proper Pasteurization of Milk? Swenarton, J. C. Jour. Bact., 1927, *xiii*, No 6, p 416

Sixteen pasteurizing plants were studied with respect to the B. coli contents of the pasteurized milk.

The B. coli content of the pasteurized milk from the different plants was found to vary considerably.

The control charts from the plants whose milk was high in B. coli show improper heating or irregularity of procedure. There is a definite correlation between B. coli content and procedure as indicated by the control charts.

A test for B. coli in pasteurized milk can be used to good advantage in checking up on plant performance.

A standard is proposed for the maximum B. coli content of a properly pasteurized milk as follows:

The standard portion of milk shall be 0.1 cc. The standard sample shall consist of five standard portions of 0.1 cc each.

Of all the standard 0.1 cc portions examined, not more than 20 per cent shall show the presence of organisms of the B. coli group.

in saturated ammonium sulphate solution, so as to obtain solutions differing in strength by 0.05 mg of quinine, the difference being made up with saturated ammonium sulphate.

At this point Row introduces a modification in the use of N/100 iodine solution as an indicator.

0.5 cc of this solution is added to each tube and the contents mixed.

It was then ascertained between which of the known solutions differing by 0.05 mg of alkaloid content the unknown lay, and then the remaining 5 cc of the quinine solution was matched against another set of test tubes containing known quantities of quinine (1 in 100,000) differing in strength by 0.01 mg between the range found in the preliminary trial, for more accurate determination.

He found that

1 Tannet's reagent does not give good results in concentrations higher than 0.025 mg in 5 cc especially when the matching tubes are heated in the brine bath and then cooled as recommended by Ramsden and Lipkin.

2 On using Wagner's reagent the solutions become colored too deep a yellow for making delicate comparisons.

3 An acidified N/100 iodine solution has been found to give very good results in detecting a difference of 0.001 mg in the alkaloid contents between the ranges of 0.045 mg in 5 cc to 0.03 mg in 5 cc and a difference of 0.001 mg between 0.03 mg in 5 cc to 0.001 mg in 5 cc concentrations.

4 With 5 cc as total volume even the presence of 0.0005 mg, i.e. 1 in 10,000,000 can be detected when matched against 5 cc of a saturated ammonium sulphate solution alone to which the same quantity of the reagent has been added.

5 With 2 cc as total volume, even the presence of 0.0001 mg, i.e. 1 in 20,000,000 can be detected provided it is matched against a blank.

**SCARLET FEVER** Amato Bodies in Scarlet Fever Toomey J A and Gammel, J A  
Am Jour Dis Child, 1927, XLIV, No 5 841

The investigations of the authors lead them to conclude that Amato bodies are not always present in scarlet fever and may be found in other diseases, although they are present more commonly in scarlet fever than in any other infection.

**PNEUMOCOCCUS** A Specific Flocculation Reaction Occurring Between Alcoholic Extracts of Pneumococci and Anti Pneumococcus Serum Jungeblut C W Jour Exper Med 1927, XL, No 2, p 227

**Antigens** The bacterial antigens were prepared by extracting the centrifugized, washed sediment of 1500 cc of an 18 hour pneumococcus broth culture in 40 cc of absolute or 95 per cent ethyl alcohol for a period of 4 weeks, at room temperature. The extracts were then filtered through paper and were ready for use. Antigens obtained by extracting the bacterial sediment for 2 hours with boiling alcohol in a reflux container were found to be only slightly weaker in flocculating power than those prepared by the first method.

To one part of benzoin tincture are added twelve parts of alcoholic bacterial antigen. This initial mixture is suspended in 0.85 per cent salt solution by adding quickly 25 cc of the salt solution to 0.4 cc of the initial mixture. It is important to add the diluent very quickly. Experiments in which buffered salt solutions of different  $P_H$  values were used in preparing this suspension indicated a neutral medium as the most desirable for the reaction. The colloidal suspension thus obtained is slightly opaque and just on the verge of spontaneous flocculation, if left standing at room temperature or shaken vigorously it will flocculate by itself. In order to insure conditions of maximum sensitivity, it is necessary to determine for each new antigen and new benzoin tincture the optimum balance between bacterial antigens, benzoin and salt solution. A constant volume of 25 cc of this suspension is mixed with varying amounts of serum, starting with

0.5 cc and ending with 0.05 cc. It is not advisable to test smaller amounts than 0.05 cc because it was found that less than 0.05 cc of normal serum is insufficient to stabilize the suspension, the comparable range of specific flocculation is thus limited to the amounts indicated. The tubes are incubated, half immersed in a water bath at 40° C since this temperature was found to provide conditions of the greatest sensitivity, maintaining at the same time the highest degree of specificity, it was observed that normal horse serum flocculated slightly above 46° C. Readings are made after ½ hour, 1 hour, and 2 hour intervals to determine the degree of flocculation. Occasionally, another reading is made on the following day.

The reaction appears to be species specific. It is not strictly type specific, as slight or moderate cross reactions occurred between Type I serums and Type II and Type III extracts.

The flocculating power of the serum from five horses undergoing immunization with pneumococcus, Type I, did not develop to any extent before the end of the 4th or 5th month.

In the case of two of these horses in which it was possible to carry out parallel tests on a larger number of subsequent bleedings until the end of immunization, some relationship was suggested between the flocculating power and the protective titer as ascertained by the routine method of standardization in mice.

#### BACTERIOLOGICAL TECHNIC A Method for the Detection of Changes in Gelatin Due to Bacteria, Frazier, W. C. Jour Infect Dis., 1926, XXXIX, No. 4, 302

The method is as follows:

The gelatin agar medium used for the test is made as follows. In 100 cc of distilled water are dissolved NaCl 5.0 gm,  $\text{KH}_2\text{PO}_4$  0.5 gm,  $\text{K}_2\text{HPO}_4$  1.5 gm.

Four grams of bacto gelatin are dissolved in 400 cc of distilled water and to this solution are added dextrose 0.05 gm, bacto peptone 0.1 gm, beef infusion 10 cc.

The two solutions are poured together, heated in a steamer and then mixed with 500 cc of 3% washed agar. The pH is adjusted to about 7.0. The medium is placed in tubes or flasks and sterilized in the autoclave.

Plates of the gelatin agar medium are poured and allowed to harden. Duplicate plates are inoculated on the surface of the agar at the center so as to form a giant colony of the organism, and are incubated for two or three days at the optimum temperature of the organism. In general, incubation for 48 hours at 30° C was found to be satisfactory for the organisms studied.

After incubation, one plate is flooded with a 1% solution of tannic acid, while the duplicate plate is flooded with an acid solution of bichloride of mercury of the following composition:  $\text{HgCl}_2$  15 gm, HCl (conc.) 20 cc, and water 100 cc.

If the gelatin has been changed, a clear zone will appear about the giant colony on the plate flooded with acid bichloride of mercury solution, surrounded by the cloudy precipitate of unchanged gelatin. The reaction is slow, and 15 to 30 minutes should be allowed for its completion.

The plate flooded with tannic acid solution will present an appearance that will vary with the amount and degree of action of the bacteria on the gelatin. An organism that acts on gelatin with little or no increase in amino nitrogen gives a white precipitate about the colony, heavier than the precipitate of gelatin throughout the rest of the plate. With some groups of organisms this precipitate is very heavy and white, with a distinct edge. If there has been considerable decomposition of the gelatin, there is a clear zone about the colony, surrounded by a distinct white ring. This reaction takes place rapidly.

A modification of Sorensen's formal titration method was devised for testing for an increase in amino nitrogen, for it was found that after the organisms had been grouped by the gelatin agar plate method they could be further subdivided on the basis of whether or not they decomposed gelatin with an increase in amino nitrogen. The

'amino  $P_H$ ' method, as it has been termed, has the advantages of requiring no standard acid or alkali and of using only 1 cc of the solution to be tested.

To 1 cc of the solution to be tested are added 5 cc of distilled water and a drop of phenolphthalein indicator. The tube containing 1 cc of the control is brought to a faint pink color that is to a  $P_H$  of about 8.4 by the addition of dilute alkali. The tubes containing solutions to be tested are then matched to the pink color of the control that is, brought to the same  $P_H$ . Then to each tube is added 0.5 cc of formaldehyde solution (about 37%) which has previously been made pink to phenolphthalein. If the pink color disappears from the control solution dilute alkali is added drop by drop until the pink color reappears. The same number of drops of the alkali are then added to each of the other tube. If the tubes are pink no increase in amino nitrogen has taken place. The  $P_H$  of the liquid in the colorless tubes is determined colorimetrically by the addition of phenol red or brom thymol blue indicator and this value is called the amino  $P_H$  number.

This method has been very useful in detecting small increases in amino nitrogen and also gives a rough idea of the amount of that increase. It is useful in testing clear synthetic media like the gelatin solution but cannot be used of course in colored solutions.

The paper is illustrated with 12 microphotographs illustrating the reactions.

**LEPTOSPIRAS** A Method for the Isolation of Leptospiras from Water Bauer J H  
Am Jour Trop Med 1927 vii No 1 p 147

The water was filtered first through paper then through Berkefeld V and finally through Berkefeld N filters. The filtrate served as the inoculum as well as a constituent of the medium used. This medium was prepared on the same principle as Noguchi's semi-solid leptospira medium with the modification that approximately 1 per cent of fresh defibrinated guinea pig's blood was used in place of 10 per cent of rabbit's serum. The various ingredients were mixed in the following proportions:

Filtered water	-----	- - - - -	- - - - -	100 cc
2 per cent nutritive agar melted and cooled to 45° C	- - - - -	- - - - -	- - - - -	20 cc
Fresh defibrinated guinea pig's blood	- - - - -	- - - - -	- - - - -	to 3 cc

This mixture was placed in a number of ordinary test tubes approximately 7 cc in each and kept at room temperature in the dark. The temperature in the laboratory varied between 22 and 35° C.

If leptospiras were present in the water growth usually occurred between the sixth and tenth day of incubation. It was clearly visible to the naked eye as a grayish haze and a partial decoloration of the hemoglobin extending about 1 cm below the surface of the medium. When examined under the dark field microscope this haze showed large numbers of leptospiras. In the examination of positive samples of water it was usual to find one or more tubes containing pure cultures of leptospiras while most of the tubes showed mixed cultures of leptospiras and other spirochetes or only several types of these other spirochetes. If the medium was placed in a large number of small test tubes about 2 or 3 cc in each most of the tubes usually contained only one kind of organism.

**SPIROCHETA PALLIDA** Demonstration of in Single Microscopic Sections Dieterle  
R R Arch Neurol and Psych 1927 viii 73

A reliable technique has been developed which has been found more satisfactory than any other method on account of its reliability, simplicity and time saving features.

1 After fixation and cutting place the preparations in a 1 per cent solution of uranyl nitrate in 70 per cent alcohol at 55° C for one half hour.

2 Wash for a moment in distilled water.

3 Pass the sections through 96 per cent alcohol U S P.



4 Handling the sections individually, place them in an absolute alcoholic solution of gum mastic (10 per cent) long enough to allow thorough infiltration—about 30 seconds Immerse section for an instant in 96 per cent alcohol

5 Transfer to distilled water

6 Silver for from 1 to 6 hours at 55° C in 1 per cent aqueous silver nitrate solution, carrying out this procedure without exposure to light

7 Wash for a moment in water

8 Develop in the following reducing solution for from 5 to 15 minutes

Hydrochinone -----	15 gm
Sodium sulphite -----	0.25 gm
Neutral solution of formaldehyde (Merck's Neutral "Blue Label")	
40 per cent solution -----	10 cc
Acetone -----	10 cc
Pyridine -----	10 cc
Water, to make -----	90 cc

Mix and dissolve these and then add 10 cc of 10 per cent absolute alcoholic mastic solution to make the mixture milky

9 Wash for a moment in distilled water

10 Dissolve out the mastic and dehydrate by transferring the sections to 96 per cent alcohol U S P, and then to acetone Clear in xylene and mount in Canada balsam

The distilled water must be free from chlorides and all glassware must likewise be clean Frozen and celloidin sections are handled with glass hooks and cover glasses with ordinary forceps

The final result produces sections that are a golden yellow to golden brown, according to thickness The spirochetes are brown to black in proportion to the heaviness of impregnation

#### GONOCOCCUS A Method for Preserving the Life of Gonococcus Cultures, Krantz, W., Dermat Wehn, 1927, LXXX, 1252

Utilizing the observation of Kaudiba that the life of gonococcus cultures may be prolonged if only a single spot on a plate is touched with the inoculating needle, Krantz makes such a "spot inoculation" on an ascites agar plate which is then inverted over the lid of a larger dish containing a bichloride solution to prevent contamination and supply moisture

A single large colony results from which transplants may be made, according to Krantz, for as long as 3 months

## REVIEWS

Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building  
Richmond Va

### *Diseases of the Skin and Syphilis\**

THE aim of this book is to present a teaching treatise on dermatology for the use of the student and general practitioner. The object sought was to so present the pathologic, the clinical and therapeutic facts as to render the clinical clear on the basis of the pathologic and the therapeutic sound on the basis of the pathologic and clinical.

The plan of the volume is thus both ambitious and practical. Tabular presentations of differential diagnosis are frequently used and in the presentation of formulas the exact purpose of each ingredient is indicated, a new and useful departure.

The volume can be recommended not only to the student but to the practitioner at large.

The book is profusely illustrated mainly with photographs which are excellently reproduced.

### *American Health Congress†*

A REPORT of the Proceedings of the Forty-first Annual Meeting of the Conference of State and Provincial Health Authorities of North America held in 1920.

Numerous papers on a variety of subjects concerned with public health of especial interest being a report of the plague situation in California, the need of epidemiologic research in trachoma, and a report concerned with the conservation of vision.

### *Annals of the "Pickett Thomson" Research Laboratory‡*

THIS publication lists the activities of these laboratories founded by Mr F N Pickett and Dr D Thomson and affiliated with St Paul's Hospital London.

The publication appears irregularly and is devoted mainly to bacteriology, protozoology, and biochemistry and to recording photographically the whole range of bacteria and protozoa.

In Volume I, No 1 are reported studies upon the etiology of measles and scarlet fever, studies of the virus of variola vaccinia, experiments with de-fatted tubercle bacilli, studies of detoxified vaccines, and the treatment of war gas poisonings and microphotographic studies of the gonococcus, meningococcus and M catarrhalis.

There are 114 microphotographs which are truly a work of art, wonderfully well selected and executed and marvelously reproduced.

No 2 of Volume I describes the preparation of special varieties of culture media, researches on B pneumosintes and on the bacteriology of the upper respiratory tract in influenza, measles, etc., relating the discovery of six new bacteria, the description of a new

*Dis. of the Skin and Syphilis.* By Albert Strickler M.D. Professor of Dermatology and Syphilology, Temple University. Cloth 629 pages, 214 illustrations. F. A. Davis Co. Philadelphia.

†American Health Congress Series. Vol III. Paper. 36 pages. National Health Council, New York.

‡Annals of the Pickett Thomson Research Laboratory. Vol I Nos 1 and 2. Paper. 29 pages, numerous microphotographs. Vol II No 1. Paper. 8 pages, 60 microphotographs. Baillière Tinsall and Cox, London.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

method for the mechanical disintegration of tissues and bacteria, and microphotographic studies of Friedländer's bacillus, influenza bacillus, *B. hemophilus hemolyticus*, *B. pertussis*, pneumococcus (all types), enterococcus, and Tammchiff's diplococcus (measles)

This section contains nearly 100 microphotographs of bacteria exclusive of other illustrations

Volume II, Part I the discovery of a new bacillus during the study of measles, four new micrococci from the respiratory tract, a study of *B. pneumonitis vaccine*, and a photographic record of certain chromogenic bacteria

In the 28 pages of this section there are 60 microphotographs of superlative excellence

The purpose of this publication is, eventually, to furnish monographs of the various pathogenic bacteria giving in epitome of the various researches upon them, and ultimately to furnish a very complete atlas of bacteria and other microorganisms recording type species photographically

If this purpose is ultimately achieved much of the difficulty now experienced in visualizing a given germ from a written description will be obviated by reference to the exceedingly valuable microphotographic records now inaugurated in these publications

It is to be regretted that so valuable a publication should be bound in paper, no other criticism is warranted

### *Annals of the Pickett-Thompson Research Laboratory\**

AS THOSE who are familiar with previous volumes of this publication know, its principal purpose is to record photographically the whole range of bacteria and protozoa. The present volume is entirely devoted to researches upon streptococci and constitutes the most comprehensive exposition of this most important, as well as very confused, group of organisms that has, as yet, been presented in one place.

Approximately half the book is devoted to a detailed résumé of numerous technical studies embracing numerous cultural and other methods of study. This portion of the book will be of the greatest interest and value to bacteriologists and laboratory workers as well, especially, the section on the biochemical reactions of the streptococci.

There are also studies of the soluble products of the streptococci, their virality, their virulence, their immunity reactions, and, among a host of other things, the classification of nonhemolytic types by the use of Crowe's medium.

The final section of the work is embellished by 79 colored plates of reactions on Crowe's medium and 306 microphotographs presented is an aid to the identification of various members of the streptococcus group.

The volume is one of great value and deserves a place in the library of every bacteriologist and laboratory worker.

### *United Fruit Company Medical Report†*

AMERICAN "Big Business" has often been accused of many things in connection with their South American interests. The report in this publication details the very constructive work of the medical department of one of the largest American industries in South America.

The first three sections of the report are concerned largely with statistical data and very informative as to the conditions encountered and the results secured in the company hospitals.

Section IV comprises comments on some of the chief tropical diseases and their treatment.

\*Annals of the Pickett-Thompson Research Laboratory, Vol. III, Paper 316, pages 306 microphotographs, 79 colored plates. Williams and Wilkins Co., Baltimore.  
†United Fruit Company Medical Department, Fourteenth Annual Report, Paper 320, pages. United Fruit Co., Boston.

Section V consists of interesting case reports and Section VI contains twenty two papers by members of the Medical Staff covering a variety of subjects and investigations. The report is of distinct interest and value.

### *A Textbook of Pathology\**

THIS fourteenth edition of a well known text will be welcomed by all those for whom it has long served as a source of reference.

For many years the reviewer has used it as such and has never failed to find some reference to the matter at hand no matter how unusual or infrequent and this has doubtless been the experience of many others.

This new edition has undergone a very thorough and comprehensive revision much new matter covering the important developments of recent years and many new illustrations have been added. The bibliography has also been considerably extended.

The sections covering poisoning and histologic technique in former editions are omitted in this as being better and more fully covered in special works. The technique of postmortem examinations excluding the preservation and staining of tissues has been retained.

The book is an excellent example of the printer's craftsmanship and will undoubtedly be as favorably received as its predecessor.

### *Diseases of the Newborn†*

IN THIS compact volume the author very ably discusses the diseases which may be encountered during the first month of life.

The book not only represents an intelligent survey of the literature but ably reflects the practical experience of the author and can be recommended as a most useful reference for both student and practitioner to whom it should come as a very welcome addition to their working library.

### *Hygiene and Sanitation for Nurses‡*

A VERY clearly written textbook for the instruction of nurses which is now in its fifth edition. Eminently satisfactory for the purpose.

### *The Health of the Child of School Age§*

IN THIS small volume are collected a series of ten lectures delivered by various authors upon various subjects before the Institute of Hygiene.

The subjects discussed are: The Dental Problem in Relation to School Children, The Prevention of Nervous Affections in the Young, Diet in Schools, Disorders of Digestion During School Life, The Incidence of Infectious Diseases, Affections of the Nose, Throat, and Ear and Their Prevention, Eye Troubles, Some Disorders of the Skin and Their Prevention, The Value of Sport and Physical Exercise and Preventable Deformities in Childhood.

The lecturers are all well known to the Medical Profession of England and the volume should be of interest and value to school physicians and pediatricians.

\*A Textbook of Pathology. By F. Delafield M.D. and T. M. Prudden M.D. Fourteenth Edition revised by F. C. Wood M.D. Director Laboratory St. Luke's Hospital New York. Cloth 1339 pages 20 full-page plates 530 text illustrations. William Wood and Co. New York.

†Diseases of the Newborn. A Textbook for Students and Practitioners. By Jane E. Burnet. Cloth 276 pages. Oxford University Press New York.

‡Hygiene and Sanitation for Nurses. By G. M. Price M.D. Cloth 86 pages. Lea and Febiger Philadelphia.

§The Health of the Child of School Age. By various authors with a foreword by Sir Thomas Oliver Professor of Medicine University of Durham. Cloth 304 pages. Oxford University Press New York.

### *Mode n Practice of Pediatrics\**

THE book is the most complete one volume work on the subject of Pediatrics that the reviewer has seen and apparently covers as much of the whole field of the subject as could be crowded into one volume.

Besides the author's own views, brief and comprehensive references are given to the important works on most of the various subjects under discussion. Each chapter is well written and there is little in the whole volume that would fail to interest the most critical reader. Particularly noteworthy is the chapter on "Breast Feeding." The author deals with its importance from numerous angles presenting brief and convincing statistics correlated in a most instructive and comprehensive sort of fashion. The chapter on "Metabolism" leaves little that is known on the subject unsaid and briefly states the views of more recent investigators of this subject.

The chapter on "Artificial Feeding" indicates a thorough knowledge on the part of the writer of the important work that has been done in this field. The writer's own views and conclusions are briefly expressed, adequate emphasis being placed on caloric requirements and the proper food elements, for both the normal and the abnormal infant. The author's feeding methods are simple and at the same time based on sound scientific principles.

Obviously the book could not be regarded as an exhaustive treatise on the subject of Pediatrics, but taken as a whole for practical purposes the field is well covered and it is hard to overestimate the value of a reference work of this kind for the student and busy practitioner.

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### *Diseases of the Heart Their Diagnosis, Prognosis and Treatment by Modern Methods†*

WITHIN recent years tremendous strides have been made in the direction of better understanding of cardiac disorders. This progress has been due in great part to the introduction of graphic methods, instruments of precision, into the examination of the cardiac mechanism. The scope of this volume, however, is not confined to a description of the usefulness of graphic methods. It includes within its compass also an account of such knowledge of the diagnosis, prognosis and treatment of cardiac disorders as was in our possession prior to the introduction of these more recent methods.

The author has purposely avoided the discussion of matters of merely theoretical interest. The phraseology is comparatively free from technical terms. The book is written for the clinician, not the specialist, and should find a useful place among the practitioner's books of reference.

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### *A Textbook of Pharmacology‡*

THIS is a textbook intended primarily for the student but of value, also, to the practicing physician.

Part I discusses the drugs ordinarily employed in the treatment of disease, Part II is devoted to various new and nonofficial remedies, and Part III includes prescription writing, drug eruptions, incompatibilities, poisons, and antidotes, etc.

The style is succinct and clear cut. The book should prove of use to the student and a handy source of ready reference for the practitioner.

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\*The Modern Practice of Pediatrics. By William Palmer Lucas M.D. LL.D. Illustrated. Cloth. Pp. 962. The Macmillan Company. 1927.

†Diseases of the Heart Their Diagnosis, Prognosis and Treatment by Modern Methods. By Frederick W. Price M.D. F.R.S. (Edin.) Cloth. Pp. 531. Oxford University Press American Branch New York.

‡A Textbook of Pharmacology. By A. D. Bush M.D. Professor of Pharmacology, University of Pennsylvania. Cloth. 182 pages. P. Blakiston's Son and Co. Philadelphia.

### *Ker's Manual of Fevers\**

THIS book is intended for the use of English students taking the statutory course of "Fevers" at an Isolation Hospital but many will be added to the working library of physicians at large

The diseases discussed are measles rubella, scarlet fever smallpox vaccinia chicken pox typhus fever, enteric fever diphtheria erysipelas whooping cough, mumps, and cerebro spinal meningitis

The text is practical well written and comprehensive within its scope

As a reference volume for the physician or a text for the student it can be recommended without reserve

### *Clinical Diagnosis†*

TWENTY years ago Dr Todd's manual supplied to the student and practicing physician an authoritative source of practical information concerning clinical laboratory methods, their application and their interpretation

That it has never relinquished the outstanding position it then assumed and that it is among the most frequently consulted reference books of practically every clinical laboratory are matters of common information. It is not surprising, therefore that it has passed through many editions and will probably pass through many more for Todd's manual is one of the books whose inherent excellence and comprehensive usefulness assures its continued existence

In this new, reset and extensively revised edition the original author is associated with Dr A H Sanford as collaborator whose fitness for such a position requires no comment

While the revision of the work has been quite general and complete, due to the omission of some little used methods and the skilful use of type the number of pages is not increased although the volume contains more matter than before

The chapter on animal parasites has been largely rewritten and that on bacteriology extensively revised

There are 35 new illustrations and many more have been replaced

As usual the typography is excellent

The present manual is quite up to date and can be heartily commended without reserve as a necessity for the student the practicing physician and the laboratory worker

### *Detection of Poisons and Powerful Drugs‡*

THAT this work has passed through six editions is a significant attestation as to its worth and usefulness

The forensic detection of poisons and drugs is a procedure demanding a high degree of skill and experience, as the study of this book amply demonstrates. To those engaged in such work it will serve as an authoritative and complete reference. For the clinical or chemical laboratory worker it should be extremely useful as a source of authoritative methods in the performance of preliminary examinations, Chapter V being a veritable mine of information in this respect

This volume, like its predecessors can be recommended without reserve

\*Ker's Manual of Fevers Third Edition revised by C Rundle Lecturer on Infectious Diseases University of Liverpool Cloth 6 plates 15 illustrations Oxford University Press New York

†Clinical Diagnosis by Laboratory Methods By James Campbell Todd Ph B M D Professor of Clinical Pathology University of Colorado and Arthur H Sanford M D Professor of Clinical Pathology University of Minnesota (The Mayo Foundation) Head of Section on Clinical Laboratory Mayo Clinic Sixth Edition Revised and Reset 748 pages with 346 illustrations 29 in color Cloth Philadelphia and London W B Saunders Company 1907

‡Laboratory Manual for the Detection of Poisons and Powerful Drugs By Wilhelm Autenreth Sixth American Edition Authorized Translation by W H Warren Ph D Professor of Organic Chemistry Clark University Cloth 694 pages 60 illustrations P Blakiston's Son and Co Philadelphia

## *Dental Infection and Systemic Diseases\**

THE assignment of dental disease to its proper place as a causative factor of systemic disease is a problem the existence of which is recognized, but the solution of which requires a large amount of persistent and systematic study.

To this problem Dr Haden's little volume brings a contribution of distinct value and definite interest to the physician, the dentist, and the laboratory worker all of whom, perforce, must be alert to the importance of an eventual understanding of this very complicated factor concerned with the diagnosis, treatment, and prophylaxis of disease.

The book records in a connected and succinct way the results of a large amount of research upon dental infections and their relation to disease which has been conducted by the author during the past six years.

After a brief historical résumé the bacteriology of chronic dental infection and the technique suitable for its study are discussed. The most striking feature noted was the very high percentage of positive cultures in radiographic negative teeth. Nine per cent of radiographic positive teeth were sterile, in opposition to the belief of some that all pulpless teeth are infected. The most common organism was the streptococcus, the type determination of which Haden believes unimportant.

The detection of dental infection is discussed. In a large series no information of value was obtained from careful studies of the leucocytes.

The types of systemic disease associated with chronic dental infection are then listed and briefly discussed.

Chapter VI is devoted to a discussion of the lesions in rabbits following the injection of bacteria from chronic periapical infection, and evidence is adduced in support of a tendency on the part of these bacteria to specific localization.

The remainder of the volume is devoted to the history of cases illustrating the relation of dental infection to systemic disease, thirty five such cases being reported in greater or less detail.

The illustrations and general typography of the volume are excellent and it can be recommended as a definite contribution to the subject.

## *Recent Advances in Biochemistry†*

THE purpose of this little volume is to present as diverse as possible a selection of topics in which the recent advances have been most striking and in which the trend of modern biochemical research is well indicated.

It is not, therefore, a textbook but a review of the more important contributions of recent years to the subject, the selections being necessarily somewhat arbitrary.

The fact that the volume has reached within a year a second edition, suggests that it has been of value to those to whom it is addressed.

The following subjects are discussed in greater or less detail: aspects of protein metabolism, colloids and the physical chemistry of proteins, nucleoproteins, the role of tyrosine, the carbohydrates, the biochemistry of fats, the role of sulphur, the vitamins, hemoglobin and related pigments, the chemical basis of specific immunologic reactions, chemotherapy.

It is obvious by the nature of the volume and the complexity of the subject that their discussion is not exhaustive. As a source of reference of the recent advances in biochemistry it can be recommended as presenting a bird's eye view of the outstanding contributions of recent times.

\**Dental Infection and Systemic Disease*. By R. L. Haden, M.D., Professor, Department of Dental Medicine, University of Kansas. With a foreword by E. C. Rosenow, M.D. Cloth 185 pages, 63 engravings. Lea and Febiger, Philadelphia.

†*Recent Advances in Biochemistry*. John Pryde, Lecturer in Physiological Chemistry, University of Wales. 2nd Edition. Cloth 370 pages, 38 illustrations. P. Blakiston's Son and Co., Philadelphia.

# The Journal of Laboratory and Clinical Medicine

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Editor WARREN P VAUGHAN MD

Richmond Va

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## EDITORIALS

### *Tweedledum and Tweedledee*

TWO physicians were in earnest consultation over the case of a woman with lobar pneumonia. Her husband was attending the discussion. In the course of conversation the family physician whom we shall call Dr N N R, remarked that the invalid had been receiving digital in stated doses. Some minutes later the consultant let us call him Dr U S P, agreed that digitalis was clearly indicated. The anxious husband eagerly alert paled as he remarked, 'But, Doctor, she has had no digitalis whatsoever.' It took some time to reassure him to the effect that digital and digitalis were synonymous terms.

The embryo physician learns his pharmacology and becomes well acquainted with the terms of the United States Pharmacopeia. When he stands diploma in hand, he has not only achieved a doctorate in medicine but he has also mastered a new language the language of scientific medical terminology which to the uninitiated is as so much Hebrew. He is happy in his knowledge and proud of his acquaintance with the Latin and Greek suffixes and



prefixes which has enabled him to readily understand the derivation of his medical terms

He opens his office The corner druggist provides him with "Come to me" prescription blanks Like as not his first visitors are a long line of detail men representing the various pharmaceutical concerns, each of whom recites his piece with apparent sophistication and with every semblance of scientific accuracy

Suddenly, like Alice, the young physician finds himself in wonderland He has placed his sample bottles on a shelf in the cabinet or the laboratory (what physician has not an untidy shelf groaning under a conglomeration of sample medicines?), and in his leisure he decides to study over the day's catch To his utter amazement he discovers that his linguistic training avails him nothing Here are words that he has never dreamed of and whose etymologie origin he finds it impossible to trace

Let's see, what came in today? Thiogen Oh, yes, something that generates sulphur Let's read further A concentrated solution of the sulphates and subsulphates of sodium potassium magnesium and calcium Ah, this has the ring of familiarity Sodium sulphate and magnesium sulphate The old lady has put on her Easter bonnet What next? Elixir of pyraminal Literal translation indicates something about fire and amines No, this is too deep Let's look farther Oh, yes, here it is It contains pyramidon and luminal The terminology mutations have been carried one step farther and the naming of pyramidon and luminal has given birth to a new species, pyraminal But we have been prescribing pyramidon and luminal mixed by the druggist and dispensed in capsules for sometime Excellent idea to disperse them already mixed It will save the druggist so much time, the new short name will save the doctor some ink and some brain work and the patient will know that he is receiving the newest and latest of the synthetic preparations Manufacturer, druggist, doctor, patient, rich man, poor man, beggar man, all will be the happier

Metatone That sounds good An alterative tonic Let's see what it contains Vitamin B extract Here's news We hadn't realized that they had perfected an extract of vitamins as yet We live and learn Nucleic acid Yes, of course, a derivative from the nuclei of cells which of course must have great tonic value Oh, yes, and here are a lot of salts, glycerophosphates Of course that would be tonic and reconstructive Haven't we a blotter on our desk which assures us that glycerin tonic is excellent? We wonder just what is the difference between glycerophosphate compound and Metatone?

Here's something interesting Ceanothum It is indicated as a blood coagulant Curious our professor of pharmacology was so far behind the times as to have failed to have told us of the great therapeutic value of Ceanothum

Cheraeol Of course, an alcoholic extract or solution of cherry The formula is printed on the bottle and it looks good as a cough mixture There appear to be at least eight ingredients It would take some time to write up such a prescription using U S P terminology We can remember the word Cheraeol much easier Yes, let's prescribe Cheraeol when the opportunity arises

Bromoval with Barhital Nothing confusing about that And the ingredients are on the label Here is a good sensible prescription Barhital with triple bromide made up into an effervescent granular powder This should be easy to take Let's prescribe it Unfortunately for this preparation, however, the name is not as euphonic as Chloral and some days later we have forgotten just how the first word should be spelled So this does not get prescribed

What's next? Diasystol To be sure this must have something to do with blood pressure but our professor of pharmacology told us positively that no drug had any permanent effect in lowering blood pressure So, out of courtesy to his memory let's pass this up

And so the list might go on The scientific erudition of the detail man is sometimes so great that we wonder why he wastes his time in such an unproductive field Occasionally in our moments of doubt we wonder how much he is laughing up his sleeve as the saying goes at the avidity with which we appear to drink in his confidential tips for getting better results

Yes, we are in wonderland How many preparations of digitals are there, each with a different name? Exactly what is the pharmacologic difference between digitalis leaves digifolin digitalone digalen digiglusin and the others? For the life of us we do not know and therefore decide to prescribe that preparation whose name we recall most easily or that preparation which is most attractively packaged or that which for one reason or another seems to be a little easier of administration

We made so bold within the last month as to write a prescription for 01 gram digitalis folia pills The patient living in a nearby city of ninety thousand inhabitants wrote back with much concern that he had been unable to find a druggist who could fill the prescription No longer does the mortar and pestle hang above the druggist's entry as a sign of his profession Too infrequently now does he even use it in the little back cupboard devoted to compounding prescriptions

Within the week we wrote a prescription for tribasic citrocarbonate A certain manufacturing chemist supplies an excellent effervescent tribasic citrocarbonate, so, to save the druggist trouble, we specified the name of the manufacturer on the prescription But the word tribasic does not appear upon the ready bottled prescription package and the druggist, a conscientious man, therefore said that he could not fill the prescription This caution is of course laudable but it is scarcely to his credit that he does not know the exact nature of the ingredients of the ready prepared packages which he is dispensing

Under how many trade names can you procure a mixture of pyramidon and some phenol barbital preparation? Wherem lies the superiority of one over the other? Tweedledum and tweedledee, you shut your eyes and take your pick

Of course, there is something to be said on the other side If milady prefers to pay a dollar for ten cents worth of rouge put up in a pretty container which will look well on her dressing table why should her esthetic sense not be also consulted in the matter of tastily prepared medicaments? The manufacturing chemist has accomplished much in the line of making drugs less

disagreeable to take and we owe him a debtor's gratitude. Many of them have also invested great sums in constructive experimental research, much of which is not directly remunerative but some of which ultimately turns out to be of distinct value to the ultimate consumer. This must be paid for out of the income from sales of drugs.

We are living in a day of exaggeration in which unusual claims are made for the most commonplace and in which old friends are scarcely recognized on account of their gaudy apparel and new names. This applies even more to other commodities, soaps, cleaning powders, underclothing, silk stockings, lead pencils, radios, and so on. As long as we are living in a day of exaggerated advertising and copyright names and enjoying it, there is little probability of an early change in the matter of drugs.

We make no plea for a revolution in drug sales methods. We only urge that when a physician prescribes mince pie for his patient whether it be made according to the recipe of Grandma Jones or Old Lady Brown he knows what are the exact ingredients of said mince pie and how he will expect his patient to react thereto.

—W. T. V.

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### *Erratum*

In Volume XIII, No. 7 April, 1928, in the article by Christina and Green, entitled "Colloidal Gold Solution," on page 680, third line should read: The indicator is prepared as follows: Dissolve 0.1 gm. of phenol red, etc.

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No. 2

## CLINICAL AND EXPERIMENTAL

### A COMPARISON OF THE AGGLUTININ AND ANTITOXIN CONTENT OF ANTIDYSENTERIC SERUMS\*

By ELLA M. A. ENLows, PH.D. AND S. C. BROOKS, PH.D., WASHINGTON, D. C.

THE agglutination technic for testing the efficiency of antidyenteric serum has been used in this laboratory for a number of years.

Three isolations of the Shiga type (*Eberthella dysenteriae* Shiga) and two of the Flexner type (*Eberthella paradyenteriae*) are employed as antigens. As controls a typhoid suspension (*Eberthella typhi*), a polyvalent serum of known titer, and normal horse serum are used. These antigens are prepared in for malinized saline solutions stored at 15° C and frequently are found to vary but little for a considerable period of time (two to four years). Since it has been shown by a number of investigators and by our own experiments that antidyenteric serum when properly prepared contains substances capable of neutralizing the toxin of certain dysenteric types, the question has arisen as to the value of continuing the agglutination test as a measure of efficiency of this antiserum.

Castellani (1901), Inomata (1913), and Baldwin and Rhoades (1925) have briefly discussed the parallelism of agglutinins and protective substances.

It is recognized that the mechanism of immunity when broadly conceived comprehends very much more than the neutralization of a bacterial toxin by an antitoxin, but clinical experience with some serums (e.g., diphtheria anti-toxin) has shown that we may use this phenomenon as an index of the efficiency of certain specific therapeutic or prophylactic serums. Nor can we dispute the evidence as to the efficiency index of antisera secured from the

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†(Accl and Accl Vecsel 1924, Aoki 193, Arkwright 1920, Castellani 1901, Dopfer 190, Dreyer 1904 et seq., Duhot and Damade 1919, Fabry 191, Gardner 1923, Hausherr 191, Hirtzfeld 1920, Przemsycki Seydel and Sierkowski 19, Loewenthal 1920, McLeod and Ritchie 1920.)

disagreeable to take and we owe him a debtor's gratitude. Many of them have also invested great sums in constructive experimental research, much of which is not directly remunerative but some of which ultimately turns out to be of distinct value to the ultimate consumer. This must be paid for out of the income from sales of drugs.

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endeavored to meet some of them by using varying doses of serum against a constant multiple of the MLD and by the use of a dried toxin which we have found to be stable for a period of over six years. This procedure, while assuring us a fair degree of certainty as to the toxin used does not of course, eliminate differences between individual animals. Throughout our work on this problem painstaking necropsies of all animal fatalities have been performed (combined with cultural methods). There is no possible way of avoiding this, if accuracy is desired when the animals used are rabbits or albino mice. With the latter mouse typhoid (*Salmonella typhi murium*) is responsible for many of the irregularities especially at certain seasons of the year.

The irregularities are not so marked when rabbits are used as the test animal. It has been very difficult to obtain mice free of mouse typhoid (Webster 1922) hence almost always variable numbers of animals had to be considered in computing the protection percentage. Naturally had more animals been used for each test the variability would have been reduced. Without discussing further the nature of the toxins produced by the growth of *Eberthella dysenteriae* Shiga we will merely state that we have been unable to separate our toxins in the clean cut style described by Olitsky and Kligler (1920). Some toxins showed in a majority of tests decided effects upon either the central nervous system or the intestinal tract and these have been designated "neurotoxin" and "enterotoxin" respectively, however, lesions were nearly always produced in both the central nervous system and intestinal tract in a certain number of tests.

#### DISCUSSION OF DATA

It is the probable therapeutic value of antidyenteric serums which we attempt to determine. To do this both the agglutinin and antitoxin titrations have been used. The former yields for the same expenditure of time and money the more definite results and if it could be shown to give correct information as to the probable therapeutic values of different serums it would naturally be preferred.

But since the toxic substances produced by the growth of the Shiga type of dysentery bacilli are apparently the chief cause of the lesions occurring in dysentery of this type, one is inclined on theoretic grounds to prefer antitoxin titration as a measure of the probable therapeutic value of antidyenteric serums. The possible existence of more than one toxin is a complicating factor, since any given technic of antitoxin titration measures the power of the serum to protect against one or the other toxin, or some one definite mixture of the two. The problem then resolves itself into a study of the following questions: (1) are agglutinin and antitoxin titrations correlated? (2) are antineurotoxic and antienterotoxic titers correlated? (3) are any of these correlated with therapeutic efficacy?

The data available are not extensive enough to yield a very satisfactory answer to these questions. But since these data are fairly extensive it has seemed worth while to make more than a cursory analysis of them. We publish the results of this analysis fully conscious that the application of statis-

TABLE I

THE AGGLUTININ TITER OF SERUM FROM GOATS INJECTED SUBCUTANEOUSLY WITH KILLED AUTOLYSED CULTURES OF *EBERTHIELLA DYSENTERIAE* SHIGA, R I 100, OVER A PERIOD OF THREE AND ONE HALF MONTHS, AND THE PROTECTIVE POWER OF THE SAME SERUMS WHEN INJECTED INTO MICE TOGETHER WITH TOXINS PREPARED BY PRECIPITATION FROM FILTRATES OF BOUILLON CULTURES (NEFROTOXIN AND ENTEROTOXIN) FOURTEEN DAYS OLD OF THE SAME STRAIN (R I 100) \*

SERUM	HIGHEST SERUM DILUTION FOR COMPLETE AGGLUTINATION WITH										PER CENT OF MICE SURVIVING AFTER	
	<i>EBERTHIELLA DYSENTERIAE</i>										INJECTION OF SERUM TOXIN MIXTURE	
	SHIGA A RI 100	SHIGA B RI 1145	SHIGA C RI 73	FLENER RI 115	FLENER RI 116	FLENER RI 116	TYPHOID LINGS) III 24				NEUROTOXIN	ENTEROTOXIN
56	0	0	0	0	0	0	0				25	0
None (normal saline)	0	200	0	0	0	0	0				0	0
Normal horse serum	1600	6400	3200	6400	400	400	0				60	70
Polyvalent Antidysenteric DI	1600	3200	800	0	0	0	0				89	72
Goat 1	1600	3200	800	0	0	0	0				86	89
Goat 2	200	200	0	0	0	0	0				100	72
Goat 3	1600	1600	400	0	0	0	0				12	67

\*The serums from each goat were tested before treatment and found not to agglutinate any of the strains listed

tical methods to so small a group of cases may prove misleading, and that it is justifiable only because of the improbability that more adequate data will be available in the near future

Table I is not susceptible to statistical analysis, but offers some evidence as to the relationship between agglutinin and antitoxin titers. In it are the results of the agglutination and antitoxin tests with four goat serums of which Nos 1 and 2 showed but very little difference in agglutinin content, Goat No 2 showing a slightly higher titer with the culture which was used for its immunization. Goat 4 was almost equal to Nos 1 and 2 against the homologous culture (Shiga 100) but dropped down in titer against HL 73. Goat 3 gave a low agglutinin titer. The toxin tests were run only against Shiga RI 100. Goat 3 showed a very good antitoxin content against both of the toxins in spite of its low agglutinin titer. Goat 4 showed very little antineurotoxin content, but fair antienterotoxin content.

From these data we may give a partial answer to the first two questions: agglutinin and antitoxin titers do not necessarily run parallel, and the relative protective powers of different serums when tested against that toxin designated as neurotoxin are not necessarily the same as when tested against that considered as enterotoxin.

TABLE II

COMPARISON OF THE AGGLUTININ TITER AND ANTITOXIN CONTENT OF 17 COMMERCIAL SERUMS TOGETHER WITH SIMILAR DATA FOR A POLYVALENT ANTIDYSENTERIC SERUM (D1) SERUMS 0.1 TO 0.005 c.c. TOXIN (SHIGA RI 5 H L 73) 8 MLD

COMMERCIAL SERUM NO	AGGLUTINATION		ANTITOXIN			
	HIGHEST SERUM DILUTION FOR COMPLETE OR NEARLY COMPLETE AGGLUTINATION		CONTROL		COMMERCIAL	
	CONTROL SERUM	COMMERCIAL SERUM	NO OF MICE	SURVIVALS IN PER CENT	NO OF MICE	SURVIVALS IN PER CENT
30	3200	1600	--	--	9	88
30	3200	1600	--	--	10	80
31	3200	3200	--	--	5	60
32	3200	3200	5	60	6	83
33	3200	3200	5	60	7	85
34a	3200	1600	6	83	7	100
34b	3200	3200	3	66	4	75
35	3200	3200	7	100	8	75
36	3200	0	3	66	6	0
37	3200	1600	3	66	1	100
38	3200	3200	3	66	5	40
39	3200	3200	3	66	5	80
40	1600	1600	3	66	5	60
41	1600	1600	3	66	3	0
42	1600	800	3	66	4	75
43	1600	3200	3	66	5	0
44	1600	3200	3	66	5	60

Foreign sample

Tables II to IV give a comparison of the agglutinin and antitoxin titers of a series of commercial serums. In considering the agglutination data the variable titer of the control (polyvalent antidyenteric) serum will be observed. This is due to the use of different antigens. It is constant, of course for each individual lot of antigen. Nonspecific clumping or settling out is to be constantly guarded against in dysentery bacilli suspensions and for this



TABLE III

SAME SERUMS SHOWN IN TABLE II, BUT TESTED AGAINST TOXIN NO 55 STYLED "ENTERO TOXIN" SINCE IT SHOWED GREATER TENDENCY TO PRODUCE LESIONS IN THE LARGE INTESTINE SERUM 0.1 TO 0.005 C.C. TOXINS 8 TO 10 M.L.D

COMMERCIAL SERUM NO	AGGGLUTINATION		ANTITOXIN			
	HIGHEST SERUM DILUTION FOR COMPLETE OR NEARLY COM- PLETE AGGGLUTINATION		CONTROL		COMMERCIAL	
	CONTROL	COMMERCIAL	NO OF MICE	SURVIVALS IN PER CENT	NO OF MICE	SURVIVALS IN PER CENT
30	3200	1600	--	--	8	75
30	3200	1600	--	--	5	40
31	6400	3200	--	--	5	80
32	3200	3200	7	100	8	75
33	3200	3200	7	100	8	50
34a	3200	1600	3	100	4	100
35	3200	3200	4	75	3	66
34b	3200	3200	6	50	5	80
36*	3200	0	6	50	1	0
37	3200	1600	2	50	5	20
38	3200	3200	2	50	2	0
39	3200	3200	2	50	4	50
40	1600	1600	3	100	4	50
41	1600	1600	3	100	5	40
42	1600	800	3	100	5	20
43	1600	3200	3	100	6	50
44	1600	3200	3	100	6	0
45	3200	1600	3	100	3	66

\*Foreign sample

reason it sometimes seemed desirable to use an antigen giving a lower titer with the specific serum. Inspection of these tables will show numerous cases in which a high agglutinin titer accompanied high antitoxic content. But a number of exceptions will also be noted. Serums 41 and 43 (Table II) have a high agglutinin titer but are deficient in antitoxin content while serum 42 (Table II) exhibits a much higher antitoxic content than would be expected on the basis of agglutinin titer.

A further insight into the extent of the correlation between agglutinin and antitoxin in titers may be gained by a somewhat more detailed analysis. For this purpose Tables II to IV are used.

For the purpose of this analysis we may in each table divide the serums into groups in which (1) the agglutinin titers of commercial and control serums are the same, (2) the commercial serum has the higher titer, and (3) the commercial serum has a lower titer than the control. For each of these groups we may calculate for both commercial and control serums the mean of the survivals in per cent and its probable error. If agglutinin and antitoxin titers are appreciably correlated the ratio of these two should be about one for group one, more than one for group two, and less than one for group three. The actual results are given in Table V, and show that only in the case of the data taken from Table IV is there probably a material positive correlation. Table II actually suggests that high agglutinin titer is associated with low antitoxin titer. Table IV is, however, more significant than the other two tables both because of the greater number of tests included, and because the antitoxic content was determined by tests of protective power for rabbits, which yield more consistent data.

TABLE IV

COMPARISON OF 30 SERUMS TESTED IN RABBITS AGAINST TOXIN NO 53 (SERUMS NOS 46 47, 48 AND 49) TOXIN NO 74 (SERUMS NOS 50 64 INCLUSIVE) TOXIN NO 83 (SERUMS NOS 65 75, INCLUSIVE), MAY 22 1927 TO JUNE 1925 THE SERUM WAS TESTED IN DOSES OF 0.001 AND 0.0005 cc AGAINST 10 MLD

SERUM NO	AGGLUTINATION		ANTITOXIN			
	HIGHEST DILUTION OF SERUM FOR COMPLETE AGGLUTINATION		CONTROL		COMMERCIAL	
			NO OF RABBITS	SURVIVALS IN PER CENT	NO OF RABBITS	SURVIVALS IN PER CENT
	CONTROL	COMMERCIAL				
46	500	800	4	50	4	75
47	500	6100	4	50	4	100
48	500	3.00	4	40	4	40
49	1600	1600	4	75	4	75
50	6400	6400	4	100	4	75
51	1000	1600	4	50	4	50
52	1600	6400	4	50	4	25
53	1600	6400	4	50	4	75
54	1600	0	4	75	4	25
55	1600	0	4	75	4	25
56	1600	3200	4	75	4	75
57	3200	800	6	100	6	33
58	3.00	400	6	100	6	33
59	3200	6400	6	100	6	66
60	6400	6400	6	66	6	66
61	6400	3200	6	66	6	50
62	6400	6400	6	66	6	66
63	6400	6400	6	66	6	50
64	6400	6400	6	66	6	16
65	3200	6400	6	100	6	66
66	3200	3200	6	100	6	50
67	6400	3200	6	66	6	66
68	6400	1600	6	66	6	66
69	6400	3200	6	66	6	0
70	6400	6400	6	66	6	16
71	6400	6400	6	66	6	83
72	6400	6400	6	66	6	0
73	6400	6400	6	66	6	10
74	6400	6400	6	66	6	33
75	6400	3200	6	66	6	83
76	6400	200	6	100	6	16
77	6400	3200	6	100	6	16
79	6400	3200	6	100	6	33
80	6400	3200	6	100	6	16
81	6400	1600	6	100	6	33
82	6400	6400	6	100	6	66
83	6400	1600	6	100	6	16

It is obvious that where only two or three serums are involved the probable error is not only very high but also has very little real significance

This method of analysis leads us to conclude that there is a slight but very erratic correlation between agglutinin titer and antitoxic content \*

It seems reasonable to inquire whether the large discrepancies which sometimes occur between the two methods are not most often due to chance errors in the antitoxin titration. If this be so the agglutinin titration may be a more accurate measure of antitoxic power than the antitoxin tests themselves when these are conducted upon any practicable number of animals and would also give a more reliable indication of the probable therapeutic value of a serum

An attempt was made to obtain more exact information by calculation of a correlation coefficient between relative agglutinin titer and antitoxic content. Although a small positive coefficient resulted yet the paucity of the data make it seem inadvisable to do more than mention the fact. At least there is no contradiction of the expectation of a positive correlation

It would be desirable to study the correlations between agglutinin titration, antitoxin tests, and therapeutic value. Only in this way could a final answer be given to the question of the relative value of the different tests.

TABLE V

A COMPARISON OF THE ANTITOXIC CONTENT WITH THE AGGLUTININ TITER OF COMMERCIAL ANTIDYSENTERIC SERUMS, EACH REFERRED TO ITS CORRESPONDING CONTROL. THREE GROUPS ARE DISTINGUISHED ACCORDING AS THEIR AGGLUTININ TITER IS EQUAL TO ( $=$ ), GREATER THAN ( $>$ ) OR LESS THAN ( $<$ ) THAT OF THEIR CONTROLS. CALCULATED FROM TABLES II TO IV WITH THE OMISSION OF THOSE SERUMS FOR WHICH EITHER AGGLUTININ TITER WAS EQUAL TO 0, OR FOR WHICH NO DATA ARE GIVEN AS TO THE ANTITOXIC CONTENT OF THE CONTROL SERUM.

TABLE USED AS A BASIS	RATIO OF AGGLUTININ TITER OF COMMERCIAL SERUM TO AGGLUTININ TITER OF CONTROL SERUM	NUMBER OF SERUMS TESTED	MEANS OF SURVIVALS IN	
			CONTROL SERUM	COMMERCIAL SERUM
II	$= 1$	8	$69 \pm 3$	$65 \pm 7$
	$> 1$	2	$67 \pm 0$	$30 \pm 21$
	$< 1$	3	$75 \pm 4$	$92 \pm 6$
III	$= 1$	8	$82 \pm 6$	$54 \pm 5$
	$> 1$	2	$25 \pm 17$	$100 \pm 0$
	$< 1$	4	$52 \pm 13$	$88 \pm 0$
IV	$= 1$	15	$70 \pm 3$	$45 \pm 5$
	$> 1$	7	$66 \pm 6$	$64 \pm 6$
	$< 1$	13	$87 \pm 3$	$36 \pm 5$

## CONCLUSIONS

The available data upon the relative agglutinating and antitoxic properties of a series of commercial antidysenteric serums have been examined with a view to determining whether agglutinin titer and protective power against neuro- and enterotoxins were equally indicative of the probable therapeutic value. This examination leads us to conclude:

1 That there is no necessary parallelism between agglutinin titer and antitoxic content as usually measured by protective power, nor between this protective power against neurotoxin as compared with enterotoxin, but

2 That a slight positive correlation exists between agglutinin titer and antitoxic power so that high agglutinin titer tends to be accompanied by high protective power.

## REFERENCES

- Acel, D., and Acel Václav, Anna. Ueber die "physiologische" agglutination des *Y* Dysenteriebazillus. *Centralbl f Bakteriologie*, Jena, 1924, *xvii*, 363-370.
- Adam, A. Antikörper im Ruhrstühle. *Centralbl f Bakteriologie*, Jena, 1918, *lxxxiii*, 3-13.
- Aoki, K. Über die agglutinatorische Einteilung von dysenteriebazillen. *Tohoku Jour Exper Med*, Sendai, 1921, *ii*, 142-158.
- Aoki, K. Eine weitere agglutinatorische Untersuchung von Paratyphusbazillen. *Tohoku Jour Exper Med*, Sendai, 1923, *iv*, 12-22.
- Arkwright, J. A. Variation in Bacteria in Relation to agglutination by Salts and by Specific Sera. *Proc of Path Soc of Great Britain and Ireland*, abstr in *Jour Path and Bacteriology*, Cambridge, 1920, *xviii*, 358-360.
- Baldwin, Horace S., and Rhoades, Dorothy R. The Protective Substance in *Pneumococcus* Pneumonia. With Special Reference to Bacteremia and Specific Treatment. *Hyg Lab Bull*, U S Public Health Serv, Washington, No 141, 1925, 43-67.
- Benians, T. H. C. A Record of an Inagglutinable Form of Shiga's Dysentery Bacillus, Experimentally derived from an Agglutinable Culture. *Jour Path and Bacteriology*, Cambridge, 1920, *xviii*, 171-176.

- Beiling, R Untersuchungen über die veränderte agglutininbildung mit Ruhrbacillen vorbehandelter Kaninchen Ztschr f Immunitätsforsch u exper Therap Jena, 1919 xxviii 246-249
- Burgdorf Richard Ueber Normalagglutinine für Ruhr und andere Bazillen Centralbl f Bakteriologie, Jena, 1925, xvi 417-423
- Castellani Aldo Ueber das Verhältniss der Agglutinine zu den Schutzkroppen Ztschr f Hyg, u Infektionskrankh Leipzig 1901 xxxviii 381-392
- Davies, Arthur An Investigation Into the Serological Properties of Dysentery Stools Lancet, London November 11 1922 pp 1009-1012
- Distaso, A, Goodall, E, and Scholberg, H A Agglutination Results with Certain Dysentery Organisms Placed Against Homologous and Heterologous Sera, Jour Path and Bacteriol Cambridge 1919 xvi 2 (1)
- Dopter, Ch Sensibilisatrice Spécifique Dysentérique dans le sérum des Animaux Vaccinés et des Malades Ann de l'Inst Pasteur Paris 1903 xix 753-760
- Douglas S R On Some Characters of the Cleavage Products of Certain Bacteria With Special Reference to Their Toxicity and Antigenic Properties Brit Jour Exper Path, London, 1921 ii 170-191
- Dreyer, Georges Discussion on Immunity Agglutination Brit Med Jour London, 1904, ii, 564-567
- Dreyer, G, and Jex Blake A J On the Agglutination of Bacteria Jour Path and Bacteriol, Edinburgh and London 1906 vi 128
- Dreyer, G and Douglas, J S C On the Absorption of Agglutinin by Bacteria and the Application of Physicochemical Laws Thereto Proc Roy Soc London Ser B 1910, lxxxii, 155-205
- Dreyer, G, and Walker E W A Observations on the Production of Immune Substances, Jour Path and Bacteriol Cambridge 1909 xii 28-38
- Dreyer Georges and Imman A C The Agglutination Curve and Its Importance in the Diagnosis of Typhoid and Paratyphoid Fevers in Inoculated Persons Lancet London 1917, cxcvii 365-369
- Dreyer, Georges and Douglas J S C The Velocity of Reaction in the 'Absorption' of Specific Agglutinins by Bacteria and in the Absorption of Agglutinins Trypsin and Sulphuric Acid by Animal Charcoal Proc Roy Soc London Ser B, 1920, lxxxiii, 168-184
- Duhot E and Damade, R Sur la Sensibilisatrice Antidysentérique Jour de méd de Bordeaux, 1910 xc 260-268
- Dünner, L Die Agglutination bei Ruhr und ruhrartigen Erkrankungen Berl klin Wchnschr, Berlin 1910 in (Juli Dec) 1184-1185
- Fabry P Sur l'agglutination des Microbes atténués Compt rend Soc de biol, Paris 1921 lxxxv 237-238
- Flin, P C Over het voorkomen van Agglutinen tegenover Flexnerbacterien in het bloodserum van normale personen in Batavia Mededeel v d Burg Geneesk Dienst Batavia 1918 No 6 (Eng ed) 111
- Gardner, A D The Principles Underlying the Standardization of Agglutinable Cultures, Lancet, London 1920 cxcvii 494-501
- Gardner, A D The Normal Limit of Agglutination for B dysenteriae (Flexner) Lancet London, 1921, cci, 1269-1271
- Gardner, A D The Agglutination of Standard Agglutinable Cultures of the Flexner Group of Dysentery Bacilli by Non-dysenteric Human Sera J Hyg, Cambridge 1923, xxi 399-405
- Hauscherr Otto Beitrag zur Frage der physiologischen Agglutination von Y Ruhrbazillen Centralbl f Bakteriologie Jena 1921 lxxxviii 95-109
- Hirzfeld L, Przesmycki, F Seydel J and Sierkowski S First Report on the Standardization of Dysentery Sera Health Conference, League of Nations Paris November 1922, p 35
- Iaomata, G Der Zusammenhang zwischen dem Antikörpergehalt und der heilenden Wirkung des Dysenterieserums Verhandl d Japan path Gesellsch Tokio 1913 iii 63-64
- Jotten, K W Die Verwendung neutralisierter antiformen Bakterienanfassungen zur Gewinnung hochwertiger agglutinierender Ruhrmünsera Ztschr f Immunitätsforsch u exper Therap Jena 1920 xvi, 267-276
- Kragel J G Ueber die Ruhragglutinine insbesondere über ihr Verhalten in Krankenserum (Giessen) 8 Jena 1911 See also Centralbl f Bakteriologie Jena 1911 lviii 48-63
- Ledingham J C G, and Penfold W J Serological Tests in Dysentery Convalescents Brit Med Jour, London 1916 i 47
- Loewenthal, Waldemar Erklärungsversuche für die Ruhragglutination durch Schwangeren serum Ztschr f Immunitätsforsch Jena, 1920 xxx 439-467
- Loewenthal, W and Berkau, F Physiologische agglutination von Y Ruhrbazillen, Centralbl f Bakteriologie 1 Abt Jena 1919 lxxxiii 314-332
- Lüdke, H Untersuchungen über die bacilläre Dysenterie Ueber aktive und passive Immunisierung, Centralbl f Bakteriologie, Jena, 1905, xxxv, 612-530, 649-660, xi, 69-79

- McLeod, J W, and Ritchie, A G The Serum Reaction in Bacillary Dysentery Observations on Agglutination, With Special Reference to the Use of Freshly Prepared Bacillary Emulsions, *Jour Path and Bacteriol*, Cambridge, 1920, *xxiii*, 217 223
- Ohtsky, P K, and Kligler, I J Toxins and Antitoxins of *Bacillus Dysenteriae* Shiga, *Jour Exper Med*, New York, 1920, *xxvi*, 19 33
- Park, W H On the Interpretation of Reactions of Agglutination Among the Bacilli of Dysentery, *Med Rec*, New York, 1903, *lxxiii*, 358 359
- Ritchie, T R On the Agglutination Reaction of the Bacilli of the Typhoid dysentery Group With Normal Sera, *Lancet*, London, 1916, *cxc*, 1257 1260
- Romm, M O, and Balaschow, A J Ueber Agglutinine im Krankenserum bei der Bacillenruhr, *Centralbl f Bakteriologie*, Jena, 1912, *lxvi*, 426 442
- Speares, J, and Debono, P P Agglutination in Bacillary Dysentery, *Jour Roy Army Med Corps*, London, 1919, *xxxi*, 430 440
- Simon, G Ueber Agglutination von Paratyphus B bei Bazillenruhr, *Berl klin Wchnschr*, 1919, *lvi*, 57-59
- Sudmorsen H J and Eagleton, A J Standardization of Anti dysentery Serum, *Lancet*, London 1921, *cci*, 1109
- Trommsdorff Richard Zur Frage der Steigerung des Agglutinititers durch grosse Blutziehungen *Ztschr f Immunitatsforsch*, Jena, 1921, *xxxi*, 379 381
- de Vecchi B, and Sarti, C Sulla presenza di agglutinine e coagglutinino verso i bacilli dissenterici nel siero di individui sani e malati di varie forme infettive, *Ann d'ig*, Roma, 1918, *xxviii*, 405 421, 556 571
- de Vecchi B, and Sarti, C Agglutinine e Coagglutinino Verso i Bacilli Dissenterici in Sieri Normali e Patologici, *Patologica*, Genova, 1918, *x* 147 150
- Webster, Leslie T Experiments on Normal and Immune Mice With a Bacillus of Mouse Typhoid, *Jour Exper Med*, New York, 1922, *xxvii* 71 96
- Yule, G Udny An Introduction to the Theory of Statistics, ed 7, London, 1924, *clxxv* 352

## OBSERVATIONS ON THE MORPHOLOGY AND MOTILITY OF FUSIFORM BACILLI\*

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WHILE the majority of investigators discredit the possibility of a genetic connection between the bacillus fusiformis dentium and the coarser type of spirochete accompanying it in normal and pathologic conditions, the question of their relationship is still frequently raised and their identity stated as a fact in some current textbooks With the exception of the observations by Tunnichiff (1923), who has reported in detail the development of spiral forms in pure cultures of the bacillus, the evidence of the identity of the two organisms has been based upon the presence of occasional nonmotile spiral forms in cultures

In view of the stimulating effect of fresh tissue on the multiplication of many varieties of spirochetes and of the observation by Tunnichiff that the addition of 1 to 2 drops of  $\frac{N}{10}$  acetic acid or  $\frac{N}{10}$  sodium hydroxide to the media favored the development of the spiral forms, it was deemed that a careful study of the morphology of the bacillus in media of varying hydrogen-ion concentration and containing fresh tissue would be of interest Several workers have reported the use of fresh tissue in their cultural experiments but I have found no reference to a careful morphologic study of the bacilli made under such conditions †

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†Recently Pratt (*Jour Inf Dis* 1927 *xli* 461) observed the growth of fusiform bacilli only when cultured in serum water containing fresh tissue

Tarozzi (1905)) failed to obtain a growth of the bacillus in a bouillon media containing fresh tissue which was incubated aerobically. He learned later, however that the culture he had used was not viable. Schmamme (1912) succeeded in growing the bacillus according to the method of Tarozzi and observed that the forms were shorter than of those cultivated in serum. Brans, Pilot and Davis (1923) in their study on the flora of male smegma and Pilot and Brans (1923) cultivating excised tonsils and adenoids cultivated the bacillus in ascites tissue broth in mixed culture. Pilot and Kanter (1923) obtained the organisms also in mixed culture from female smegma in ascites tissue agar, and Tunncliffe (1923) states the presence of fresh tissue did not appear to enhance the growth of the bacillus.

*Source of Culture*—The strain of fusiform bacillus used in the present morphologic study, employing the medium of varying hydrogen ion concentration, was isolated from a case of chronic Vincent's angina by Mr. Austin Wakeford at the Thomas W. Lister Museum and Dental Institute, University of Pennsylvania. In serum agar tubes containing fresh tissue it frequently formed the typical colony extensions (Fig. 1) as noted by several observers including Leulowicz (1903, 1906) who was the first to obtain a pure culture of the bacilli. Ellerman (1906) and Krumwiede and Pratt (1913).

An interesting colony formation of the bacilli from the pure culture was observed in a slide preparation incubated at 37° containing a small quantity of macerated fresh tissue in serum agar. The preparation should be practically free from air bubbles and the cover slip sealed with high melting point paraffin. The colonies consist mostly of organisms growing out in filaments from a central area. They increase gradually in size for forty eight to seventy two hours after incubation. Using a low per cent of agar, more isolated single forms appear but the granules are not usually visible under dark field illumination during the period of active growth. Upon continuous observation under dark field illumination dividing forms in which the granules were visible were occasionally seen in the slide preparations incubated in a micro stage incubator. The line of division extended diagonally from one side of a bacillus near one granule to the opposite side near another granule thus forming the characteristic pointed ends of the bacillus.

The change in the position of the divided bacilli explains some of the arrangements one frequently observes in preparations. In some instances, after the breaking apart, the outer end of one bacillus moved slowly to one side, its newly formed pointed end appearing to remain in contact with the other organism forming an angle. In other instances after the separation, one bacillus slipped gradually beside the other forming the parallel arrangement.

*Media Used*—Two series of fluid media were prepared in vaseline sealed tubes (20 by 15 cm). The one series containing hormone bouillon at hydrogen ion concentrations ranging from  $P_H$  6.8 to 8.4 with and without fresh rabbit kidney tissue with and without glucose (0.5 per cent) and with and without inactivated horse serum (4:1). In the other similar series Hartley's broth (1922) was substituted for the bouillon and chick embryo for the kidney tissue.



Fig 1—Characteristic colony extensions of the bacillus fusiformis dentium are shown in the upper isolated colonies

*Examination*—Two tubes of each variety of media were planted one being examined almost daily for a period of two weeks the other at longer intervals. All however were kept under observation for two months.

The following Zeiss microscopic equipment was used for all examinations. Siedentopf change over condenser objective (X) 'Bitum' binocular tube attachment with compensating eyepieces Kbi 12.5X and 'Tip up' type of micro lamp or arch light.

Preparations stained vitally with brilliant cresyl blue and smears stained with Giemsa, Gram carbol gentian violet and dilute carbol fuchsin were also frequently made.

*Result*—While the best growth of the bacilli occurred in both series of media with P<sub>H</sub> 7.3 to 8.4 containing serum glucose and tissue and the least in the media without these additional substances a varying amount of growth with acid but no gas production took place in all.

Forms differing greatly in length and in number of visible granules were present and even though the granules cannot always be distinguished in the dark field, they usually become visible when carefully stained with dilute carbol fuchsin\*. An occasional long wavy form was seen but such forms are also common in cultures of other organisms which have a tendency to grow out in filaments. No indication of spirochetal forms developing from the granules could be detected at any time.

While the bacilli in the preceding pure culture study appeared to be slightly flexible they exhibited no motility. In this connection however, I would like to report the interesting observation of very motile fusiform bacilli together with nonmotile forms in slide preparations made directly from human infections upon three occasions and in two instances in mixed culture for several generations. The material for the direct examinations was obtained from a discharging ear† a pyorrhea alveolaris infection and a Vincent's angina case. Other organisms including cocci, the thicker motile type of spirochete and some of the thinner varieties were present in all three cases, also motile spirilla sputigenum in the last two and an anaerobic curved non spore bearing rod was isolated from the Vincent's angina which will be described in a future report.

From the three cases referred to above careful cultural experiments were made only with material obtained from the Vincent's infection. Cultures were made in deep tubes in various liquid and agar media with vaseline seal, with and without fresh tissue and in Schereschewsky's (1912) coagulated horse serum. A few nonmotile fusiform bacilli were seen in the mixed cultures in most of the media but motile fusiform bacilli were present only in the coagulated serum media. The implantation in this media had been made on one side of the tube approximately two inches below the surface. No vaseline seal was used but the cotton stopper was paraffined. After four or five days' incubation the media above the inoculated area had become slightly

\* I later observed that all the bacilli even in young cultures contained one or more granules which could be readily demonstrated with MacNeal's (1922) Tetrachrome stain using the phosphate buffer of Pg 64 recommended by Scott and French (1931). For best results the preparation should be fixed with heat before staining.

† The laboratory examination of this case was reported in Arch. Derm. and Syph., 1935, xv, 63.



liquefied, the fluid spaces, viewed under dark-field illumination, were filled mostly with cocci, short motile bacilli and motile spirilla, while the more solid coagulated portions from the upper part of the medium contained the greater number of very motile Vincent's, microdentium and macrodentium types of spirochetes, nonmotile filaments varying in length, together with motile and nonmotile fusiform bacilli. Most of the filaments are evidently long forms of the fusiform bacilli since many contain granules with tetrachrome stain. In the lower part of the tube which showed no macroscopic change so far, only cocci could be seen.

Transplants using the coagulated particles were made to the same media in the same manner as in the primary cultures except that a vaseline seal was used.

Within seven days the area around the inoculated site had become light gray but not much liquefied. Numerous motile spirochetes, mostly of the macrodentium type and many motile and nonmotile fusiform bacilli together with other contaminants were again present in the upper coagulated portions. The media at the bottom of the tube again showing no macroscopic change contained however, a good number of both motile and nonmotile fusiform bacilli together with only cocci. The hydrogen-ion concentration of the media at the bottom was  $P_H$  7.5 and that at the top  $P_H$  7.0.

Hoping to obtain a culture of the fusiform bacilli with possibly only the cocci contaminant, several subcultures from the material at the bottom of the tube were made to liquid serum media with fresh tissue and to the coagulated serum. Even after prolonged incubation, however, no macroscopic change occurred in the coagulated media and upon examination only a few cocci could be seen. A few nonmotile fusiform bacilli and cocci were present in the liquid tissue media. After the column of the coagulated medium containing the numerous contaminants at the top has been broken by inserting the capillary pipette for examination, the contaminants quickly spread and multiply throughout the tube, liquefying the media and producing a disagreeable odor.

The culture material for the second mixed culture of the motile fusiform bacilli was obtained from the spongy gum tissue surrounding an infected, decayed tooth. While the various types of spirochetes, nonmotile fusiform bacilli and motile spirilla were present, no motile fusiform bacilli were observable in the dark-field examination of the fresh preparation. In the cultural study, however, motile fusiform bacilli again appeared but only in the Schereschewsky's media. Instead of attempting to purify the culture as in the first experiment, a small quantity of the culture from the upper part of the tube containing more of the contaminants together with material from the bottom containing the motile fusiform bacilli, was used in making the transplants. In this way the motile fusiform bacilli were cultivated for five generations but failed to grow in the sixth transplant.

The characteristic movement of the motile fusiform bacilli can be observed best in the cultures. It is usually a darting progressive movement in either direction and impresses one as being of a more purposeful nature than that exhibited by other varieties of motile organisms. It is of a much less

vibratory type than that of the spirillum sputigenum. Occasionally a bacillus and also a longer thread form with a slower yet distinctly characteristic motion are seen. In the coagulated media the motile bacillus is not as a rule in constant motion and when at rest it is indistinguishable from the nonmotile types. To one accustomed to the nonmotile forms only the sudden movement of the motile forms through the field piercing the coagulated particles with their sharp ends impresses one as being an unusual phenomenon. In preparations made from the coagulated serum cultures motile fusiform bacilli were still present after two hours but no definite tests were made to determine how long the motility would be retained in the slide preparation.

While some of the earlier writers claim to have observed varying degrees of motility of the bacilli in material taken directly from the seat of the disease, all of the more recent observers with the exception of Stitt have noted only nonmotile forms in fresh preparation and in mixed or pure cultures.

In view of my personal observations I will briefly review the statements of authors who have observed the bacilli to be motile. Beinheim (1898) describing the organisms present in necromembranous stomatitis states that the bacilli are motile like the spirochetes. Letulle (1900) observed the bacilli in Vincent's angina to be motile if mounted in saliva. Sobel and Herman (1901) report their observations to be in accordance with those of Letulle. Carnot and Fournier (1901) found the bacilli very motile if kept at the proper temperature. Graupner (1902) states the bacilli are very motile but relinquish their activity within twenty minutes in a slide preparation. He also demonstrated a flagellum on either end and two on each side. Brion (1902) observed a slow swinging movement of the bacilli in a hanging drop. Vincent (1905) states that while the bacilli are usually nonmotile, he has observed some with slight motility in exudates but none in cultures. He also distinguishes the fusiform bacilli from the spirillum sputigenum because of the difference in their form, dimension, motility and staining reaction. Veszpremi (1907) found the bacilli to be actively motile in fresh preparations but not in cultures. Lerner (1907) noted a distinct and fairly active to and fro movement in a hanging drop. Plaut (1907) observed an unmistakable rellating motion and also demonstrated flagella by staining. Costa (1909) describes in more detail than the previous observers the character of the motility of the bacillus found in lesions which have not been exposed to the air. Stitt (1923) states "I have noted a sluggish but distinct motility when the material was mounted in saliva." The many other earlier writers and practically all the later investigators including Tunnell (1906) Larson and Brion (1913) Krumwiede and Pratt (1913) and many others however consider the bacillus nonmotile, Krumwiede and Pratt stating "The motility of the spirochetes and the lack of motility of the fusiform bacillus form the strongest evidence against their identity." Various explanations have been offered to account for the motility observed by the earlier workers. Ellerman (1905) and Muhlen (1907) who cultivated both the motile spirillum sputigenum and the nonmotile fusiform bacilli in pure culture, thought that the motile bacilli observed by others were the spirilla. Larson and Brion, and Goadby (1922) express the possibility of the reported motility being due to the presence of the motile spirochetes. In

view of the handieaps in their methods of investigation and of the fact that the distinction between the motile spirillum and the fusiform bacillus was not generally recognized at that date, it is quite probable that mistaken identifications were made

In the present study, however, with the dark-field equipment previously mentioned, both the morphology and characteristic motility of the bacilli could be quickly and beautifully demonstrated. While a long, coarse and frequently curved flagellum of the spirillum sputigenum is visible in some instances in the dark-field, no definite evidence of flagella could be seen on the motile fusiform bacilli. Several differences in the characteristics of individual strains of fusiform bacilli have thus far been generally recognized. The only variation noted by Krumweide and Pratt among the numerous strains they isolated was in the ability of some strains to ferment saccharose while others did not. Stitt recognizes two morphologic types, one which stains uniformly, the other showing the dots. Knorr (1922) describes three types differing among each other in colony formation, and Varney (1927) identified four serologic types of the bacilli and also distinguishes morphologic types. The motile bacilli I have observed are more of the longer than the shorter type which are most frequently present under normal conditions.

The real significance of the motile type, that is, whether it represents a distinct variety differing in cultural and possibly pathologic characters from the nonmotile type of the bacillus fusiformis dentium or whether the two are identical, the motility appearing only under certain conditions, must await further investigations. It would seem from my limited observations that the motile forms are more frequently present in more acute pathologic conditions since I have found no motile specimens in material taken from several normal oral cavities or extirpated tonsils, or in cultures in the Schereschewsky's media made from these sources.

In my future investigations I hope to obtain further cultures of the motile type and to study the relationship existing between the motile and nonmotile forms.

#### SUMMARY

1 No evidence of spirochetal forms was seen in a pure culture of fusiform bacilli grown anaerobically in media of varying hydrogen-ion concentration containing fresh tissue.

2 Colony formation and the division of fusiform bacilli were observed in a micro slide preparation.

3 Motile fusiform bacilli, together with nonmotile forms were found in material taken directly from pathologic conditions and in mixed cultures in Schereschewsky's coagulated horse serum media.

#### REFERENCES

- Baron Arch f Kinderh, 1902, **xxv**, 151  
 Bernheim Centralbl f Bakteriol, 1 abt, 1898, **xviii**, 177  
 Brans, Pilot, and Davis Jour Infect Dis, 1923, **xxvii**, 159  
 Carnot, and Fournier Compt rend Soc de biol, 1901, **lvi**, 143  
 Costa Compt rend Soc de biol, 1909, **lxvii**, 866  
 Ellerman Centralbl f Bakteriol, 1 abt, 1905, **xxxviii**, 383  
 Goadby Diseases of Gum and Oral Mucous Membrane, 1922

- Graupner Munchen med Wehnschr 1902 xlix 727  
 Hartley Jour Path and Bacteriol 1922 xxi 482  
 Kaor Centralbl f Bakteriolo 1 abt 1922 lxxvii 4  
 Krumwiede and Pratt Jour Infect Dis 1913 xii 199  
 Krumwiede and Pratt Jour Infect Dis 1917 xiii 438  
 Larson and Baron Jour Infect Dis 1913 xii 429  
 Leiner Centralbl f Bakteriolo 1 abt 1907 xliii 7  
 Letulle Presse Medicale 1900 vii 411  
 Lenkowiez Przegląd Lekarski 1903 xlii 107  
 Lenkowiez Centralbl f Bakteriolo 1 abt 1906 xli 1 3  
 MacNeal Jour Am Med Assn 1922 lxxviii 1192  
 Muhdens Centralbl f Bakteriolo 1 abt 1909 xliiii 523  
 Pilot, and Brans Jour Infect Dis 1913 xxi 134  
 Pilot and Kaatner Jour Infect Dis 1923 lxxvii 204  
 Plaut Centralbl f Bakteriolo 1 abt 1907 xlii 310  
 Schereschewsky Deutsch med Wehnschr 1912 xi 1335  
 Scott and French The Military Surg 1924 li 229  
 Shumamine Centralbl f Bakteriolo 1 abt 1919 lvi 311  
 Sobel, and Herman New York Med Jour 1901 lxxvii 1037  
 Stitt Practical Bacteriology 1923  
 Tappezz Atti d r Accad d Sissocrit di Siena 1905 4 8 xviii 105  
 Tunnichiff Jour Infect Dis 1906, iii 148  
 Tunnichiff Jour Infect Dis 1923 lxxviii 147  
 Veazprema Centralbl f Bakteriolo 1 abt 1907 xlii 648  
 Varney Jour Bacteriol 1927 xiii 275  
 Vincent Compt rend Soc de biol 1905 lvi 499

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## THE SO CALLED CHEMICAL TEST IN BLOOD FOR SEX DIFFERENTIATION\*

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ACCUMULATED evidence has indicated that in the blood stream of animals may be found chemical units produced by the individual organs of internal secretion. On this basis the chemical test for sex differentiation is not only an attractive idea but also very possible of solution.

A simplification of the Manoilov technique based on a study of the functions of the several reagents used by him has already been presented in a paper from this Laboratory.<sup>1</sup> It has been found necessary to devise a procedure for a more quantitative recording of the test reaction. In the original method, the end result of a test on an extract of male origin, resulted in a colorless solution of female origin, in solutions ranging in color from pink to red or violet.

In the above mentioned study it was shown that in the modified test the reaction color obtained was due to the formation of a "red acid" which could be prepared by adding a solution of potassium permanganate to an acid solution of pararosaniline hydrochloride. This "red acid" is insoluble in acids but soluble in alkali from which it can be reprecipitated by acid. Standards were prepared with this "red acid" which approximated very closely the colors

obtained in the revised reaction and were similar also to the colors obtained in the original Manoirov reaction. These color standards were prepared in the following manner: To 4 cc of water in each of 12 tubes from 1 to 12 drops of a 0.1 per cent solution of the "red acid" in 1 per cent sodium hydroxide were added, followed by 10 drops of a 1 per cent solution of gelatin and 3 drops of 20 per cent sulphuric acid. The gelatin acts as a protective colloid which prevents the reprecipitation of the "red acid" by the sulphuric acid. The depth of color depends upon the amount of "red acid" present and was denoted by a number corresponding to the number of drops of "red acid" used. The standard tubes numbered 1 to 5 inclusive ranging from colorless to but a faintly colored mixture may be taken to represent the male half of the scale; the tubes numbered 6 to 12 the last tube with a solution very deep brownish red in color, the female half of the scale.

**Examination of Blood Samples**—The blood samples were tested as follows, in accordance with the revised technique. To 3 cc of a 5 per cent solution of citrated whole blood were added in succession 1 drop of a 1 per cent solution of pararosaniline hydrochloride in 95 per cent ethyl alcohol, 35 drops of 0.02 M potassium permanganate (from a burette to get uniform drops), 3 drops of 20 per cent sulphuric acid and 10 drops of 2 per cent sodium thiosulphate. The samples of male blood were tested as well by the Manoirov reaction, Modification III)<sup>2</sup> and were found to closely parallel in color reaction the revised technique.

The summary of our findings is given in Table I. For both the male and female groups the samples as measured distribute themselves in approximately equal numbers between the male and female half of the scale. The sex of the donor, therefore, has no influence on this blood reaction. This conclusion is applicable with equal force to the original Manoirov reaction.

The examination of urine by the same procedure suggested itself. The following technique was adopted for the urine examination. To 3 cc of urine were added 2 drops of a 1 per cent solution of pararosaniline hydrochloride in

TABLE I

COLOR VALUE REVISED TFST	NUMBER OF TFSTS								TOTAL TFSTS
	AGF 11 TO 30		ACF 31 TO 40		ACF 41 TO 50		AGF 51 UP		
	FFMALE	MALE	FFMALE	MALE	FEMALE	MALE	FFMALE	MALE	
0	5		4		2				11
1	2								2
2	4		3		2		1	1	11
3	8		4	1	2		1		16
4	8	2	9	1	3	3	2		27
5	6	1	6	1	1	1			16
6	4		8	1	2				15
7		2	3	1	2				11
8	1		7	2	2	1	2		13
9			1	3		1		1	6
10	3		6		1		1		11
11			2						2
12	3		3			1	1	1	9
12+	2		3						5
Totals	49	4	57	10	17	7	8	3	155

95 per cent alcohol 45 drops of 0.02 M potassium permanganate (from a burette), 5 drops of 20 per cent sulphuric acid, and 10 drops of 2 per cent sodium thiosulphate.

The urines of more than 100 women were tested at different times in many instances different samples were secured from the same individual. The color values were found to vary for the urine specimens of different individuals and also for different samples of the same individual. These variations covered the whole range of color of the standard tubes. Similar results were secured on male samples. Table II gives a typical result for one day on a male subject.

TABLE II

TIME	SPECIFIC GRAVITY	COLOR VALUE REVISÉ TEST
9 A.M.	1.030	10
10	1.0	3
11	1.016	0
1 P.M.	1.019	0
2 P.M.	1.025	3
3	1.031	12
4	1.033	12+
5	1.030	12

The sex specificity of the Manólov reaction has already been disavowed by several workers. Riddle and Reinhart<sup>3</sup> based on a study of pigeon blood read into this reaction a correlation between it and metabolic rate. Chemically considered, the procedure involves an oxidation process which in some samples of biologic fluid is permitted to go on to completion and others is inhibited at points along the line of completion. It is probable that no single chemical substance is required for playing the part of inhibitor in this reaction. It is unlikely that we are determining the presence of the same substances in our tests on urine and blood. The chemical reactions involved in this procedure are interesting and worthy of further study. Until such studies have been made and more specific knowledge of the chemistry of this reaction is available it will be unsafe to correlate with the findings in this reaction measures or features of major biologic reactions or characters.

## REFERENCES

- <sup>1</sup>Falk, K. G. and Lorberblatt, I. *Brit Jour Exper Biol*, 1927, iv, 305.  
<sup>2</sup>Manólov, E. O. *München med Wehnschr* Dec 19, 1924, 712.  
<sup>3</sup>Riddle, O. and Reinhart, W. H. *Proc Soc Exper Biol and Med* 1927, xxiv 359-362.

## THE DISTRIBUTION OF CALCIUM IN JAUNDICED AND ACHOLIC DOGS\*

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THE fact that a deficiency in calcium in patients that are jaundiced, causes a prolonged coagulation time has long been observed. Lee and Vincent<sup>1</sup> have noted a prolonged coagulation time of the blood in patients who were jaundiced over a period of at least five weeks. These authors reduced the coagulation time of the blood in jaundiced patients in vitro as well as in vivo by the addition of calcium salts to the blood.

Grove and Vines<sup>2</sup> have shown that in the circulating blood, of the normal individual, calcium is present in two forms, namely, an ionized and a combined.

Klinke<sup>3</sup> believes that serum calcium is partly ionized (about three milligrams per hundred cubic centimeters) partly dissolved as a complex or molecular salt. The ionized calcium is ultrafiltrable and will pass through a colloidion membrane.

We have then in the blood two forms of calcium, one a combined or nondiffusible and the other an uncombined ionized salt that is diffusible.

King and King<sup>4</sup> report that in a series of seven cases of jaundiced patients the ultrafiltrable or diffusible calcium is below normal.

King and Stewart<sup>5</sup> state that in jaundiced dogs there is a 17 to 20 per cent increase in the serum calcium. King, Bigelow and Pearee<sup>6</sup> report a higher serum calcium content in the blood of jaundiced dogs than in the blood of normal dogs. Davidson and Emerson<sup>7</sup> report an increase in serum calcium following the injection of whole bile into the femoral vein of a dog.

We have a definite paradox, a lowering of diffusible calcium in jaundice, the fact that the addition of calcium salts to the blood of jaundiced patients will definitely shorten the coagulation time, and the reports of three authors who state there is a definite rise in serum calcium in jaundice. This may be explained as follows. In experimental work completed, unpublished data, we have shown that following ether anesthesia there is a very definite rise in serum calcium. King and Stewart<sup>5</sup> do not state whether blood was obtained for serum calcium before or after ether anesthesia. King, Bigelow, and Pearee<sup>6</sup> state that a week after ligation of the common duct their animals were bled to death under ether anesthesia. Davidson and Emerson<sup>7</sup> do not definitely state whether blood was obtained for serum calcium before or after ether anesthesia. However, having done this work we know that the blood for the serum calcium determinations was obtained following ether anes-

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This work was suggested and done under the direction of Dr. John J. Morton, Professor of Surgery.

thesis. This would account for the rise in serum calcium following the injection of whole bile and the rise in serum calcium in jaundice.

In all of the work, as reported in this paper, care was taken to obtain the blood for the calcium determinations before any anesthesia was given.

Kirk and King<sup>4</sup> report calcium in plasma in normal dogs under ether anesthesia was 85 mg. This is a higher figure than we obtained. However, here again the difference could easily be explained because of the anesthesia.

Cantarow, Dodak and Gordon<sup>5</sup> note quite a wide variation in the whole blood calcium in normal patients, the variation being 64 mg to 96 mg. We noted the same variation in the whole blood calcium of normal dogs, the variation being 43 mg to 70 mg.

King, Bigelow and Pearce<sup>6</sup> have noted that in dogs jaundiced a week there was a rise in the calcium content of the blood, kidneys, and feces with a decrease in the calcium content of the bone and heart. These findings do not correspond with those we obtained. The difference may be explained by the fact that their dogs were jaundiced only a week while our dogs averaged forty days each. In a week complete calcium balance would not be obtained.

Corley and Dennis<sup>7</sup> give the following results for the calcium content in normal dog's tissue: liver 264 mg, lungs 408 mg, and kidney 400 mg. These figures check very nicely with our results. We used their method for determining calcium in the tissues.

#### METHOD

Five normal dogs of as near the same weight as possible were obtained. They were placed on a mixed feed consisting of ground scraps from the hospital and containing meat, bread, bone, fruit, vegetables, and pastry. Their normal serum calcium, whole blood calcium, diffusible calcium and spinal fluid calcium were obtained according to the methods of Clark and Collip<sup>10</sup> and Moritz.<sup>11</sup> On one dog samples of blood were taken on ten consecutive days at various times of the day and the serum calcium determined. These dogs were bled to death under ether anesthesia, care being taken to obtain the blood for the calcium determinations before the anesthesia was begun. In order to remove blood from the tissues, the dogs were perfused intravenously with a 0.6 per cent sodium chloride solution, the rate and volume being equal to the rate and volume of the blood passing out. Their organs were removed, dried to constant weight, and the calcium content determined according to the method devised by Corley and Dennis.<sup>7</sup>

Three other dogs were obtained of as near the same weight as possible. Their normal serum calcium, whole blood calcium and diffusible calcium were determined. In each dog the common duct was ligated in two places and a piece of the common duct excised between the two ligatures. Each week until the animals showed signs of severe intoxication, blood was taken for the determination of serum calcium, whole blood calcium and diffusible calcium. After the dogs had been jaundiced thirty three, forty and forty nine days respectively, they were bled to death under ether anesthesia, their tissues perfused, their organs removed, dried to constant weight and the calcium content determined.



Three more dogs of as near the same weight as possible were obtained. Their normal serum calcium, whole blood calcium, and diffusible calcium were determined. In each dog a cholecystonephrostomy was done according to the method of Kapsinow, Engle and Harvey.<sup>1</sup> The gall bladder was sewed, after an opening had been made in the fundus, into the kidney pelvis. The common duct was ligated in two places and a piece of the common duct excised between the two ligatures. This produced a bile fistula, the bile passing out with the urine. Each week until the animals were killed, blood was taken for the determination of serum calcium, whole blood calcium, and diffusible calcium. At the end of seventy-eighty-four and eighty-four days respectively, the dogs were bled to death under ether anesthesia, then tissues perfused, then organs removed, dried to constant weight, and the calcium content determined.

In Dog 8, a jaundiced dog, and Dog 10, a bile fistula dog, x-rays of the pelvis and femurs of each animal were obtained at the beginning of the experiment and just before the animals were killed. The distance, voltage and milliamperes seconds on the x-rays taken at the beginning of the experiment and at the completion of the experiment were the same. The films were developed the same length of time in each case. The densities of the pelvic bones and the femurs were obtained with a photometer.

To determine whether or not some of the calcium in the tissues might be washed out with the perfusing solution a normal dog was obtained and, under ether anesthesia, the renal artery and vein of one kidney were clamped off. The renal artery and vein on the other side were left intact. The dog was bled to death, the tissues were perfused, the two kidneys removed, dried to constant weight and the calcium content determined.

The feces of two normal dogs, two jaundiced dogs, and two bile fistula dogs were obtained on three consecutive days. These feces were dried to constant weight and the calcium content determined according to the method of Corley and Denis.<sup>2</sup>

## RESULTS

The diffusible calcium of the blood, the calcium content of the blood serum, whole blood, spinal fluid, brain, liver, spleen, muscle, bone, subcutaneous tissue, heart, thyroid, gall bladder, lung, kidney, and adrenal was determined in the blood and tissues of five normal dogs. The results are summarized in Table I.

The calcium content of the blood and tissues enumerated above was determined on three jaundiced dogs. The results are summarized in Table II.

The calcium content of the blood and tissues as enumerated above was determined on three dogs having a bile fistula. The results are summarized in Table III.

Chart 1 shows the increase and decrease of the calcium content of the tissues in jaundiced and bile fistula dogs when compared to the normal.

In jaundiced dogs there is an increase in the calcium content of the muscle, thyroid, and adrenals and a slight increase in calcium content of the bone and heart. There is a marked decrease in the serum calcium, the whole

TABLE I

DOG NO	WT OF DOG	SERUM	WHOLE BLOOD		DIFFUSIBLE	SPINAL FLUID	BRAIN	LIVER	SPLEEN	MUSCUL	BONE	UPCUTANEOUS TISSUE	HEART	THYROID	LUNG	KIDNEY	ADRENAL												
			Calcium per 100 gm																										
			Calcium per 100 c c																										
Kt.	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg												
1	13.6	12.1	6.1	4.2	9.6	52.9	18.61	34.87	15.77	9.703.00	29.04	19.61	30.11	11.48	41.06	35.92	60.43												
2	10.9	13.2	5.5	5.1	7.4	47.93	23.81	64.71	17.94	11.914.60	11.03	22.07	36.74	36.42	44.54	30.07	71.63												
3	11.3	12.2	4.3	5.8	7.3	63.54	27.40	01.01	10.75	17.642.60	17.28	13.13	41.94	30.95	39.06	32.97	59.42												
4	10.9	13.2	7.0	5.4	7.2	60.57	22.26	39.70	23.18	15.311.10	24.38	25.08	44.93	37.42	14.78	34.92	61.31												
5	11.3	12.6	6.9	5.6	8.6	60.40	27.03	30.06	20.68	14.112.20	27.70	19.61	32.62	39.61	42.63	37.80	73.42												
Av	11.6	12.0	5.9	5.2	7.8	60.37	23.02	50.07	17.72	13.613.10	21.83	22.02	37.30	37.18	42.38	36.03	61.04												

TABLE II

DOG NO	NORMAL WT OF DOG	FINAL WT OF DOG	DURATION EXP	NORMAL WHOLE BLOOD		FINAL WHOLE BLOOD		NORMAL DIFFUSIBLE		FINAL DIFFUSIBLE		LIVER		SPLEEN		MUSCLE		BONE		UBCUTANEOUS TISSUE		HEART		THYROID		LUNG		KIDNEY		ADRENAL	
				Calcium per 100 cc		Calcium per 100 cc		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm	
				Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg
6	12.5	8.8	31	11.4	8.9	6.3	4.0	4.8	1.6	3.67	2.084	26.30	28.65	16.611.01	9.11	22.93	48.32	44.71	48.57	44.33	70.84	48.32	44.71	48.57	44.33	70.84	48.32	44.71	48.57	44.33	70.84
7	15.4	12.2	40	11.5	10.4	7.0	4.0	4.6	1.3	36.40	30.10	49.08	38.57	13.722.18	10.84	29.11	50.61	50.62	40.11	40.11	71.03	50.61	50.62	40.11	40.11	71.03	50.61	50.62	40.11	40.11	71.03
8	10.0	7.4	49	12.1	8.8	4.4	3.2	4.2	8	29.40	24.44	34.84	32.40	17.679.05	10.68	30.70	32.42	49.13	47.02	18.72	94.09	32.42	49.13	47.02	18.72	94.09	32.42	49.13	47.02	18.72	94.09
Av	12.9	9.4	40	11.6	9.3	5.9	3.7	4.5	1.2	32.87	27.12	36.40	33.20	10.004.90	10.20	27.70	40.41	48.21	48.03	31.37	79.87	48.21	48.03	31.37	79.87	48.21	48.03	31.37	79.87	48.21	48.03

TABLE III

DOG NO	NORMAL WT OF DOG	FINAL WT OF DOG	DURATION EXP	NORMAL WHOLE BLOOD		FINAL WHOLE BLOOD		NORMAL DIFFUSIBLE		FINAL DIFFUSIBLE		LIVER		SPLEEN		MUSCLE		BONE		UBCUTANEOUS TISSUE		HEART		THYROID		LUNG		KIDNEY		ADRENAL	
				Calcium per 100 cc		Calcium per 100 cc		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm		Calcium per 100 gm	
				Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg
9	11.0	7.7	70	11.0	14.8	1.3	10.1	4.8	7.4	49.06	29.12	60.77	20.03	13.470.46	14.99	18.06	61.86	32.40	42.70	37.96	74.90	61.86	32.40	42.70	37.96	74.90	61.86	32.40	42.70	37.96	74.90
10	9.5	10.0	84	10.8	14.2	4.8	8.4	3.4	6.8	63.41	24.60	45.73	21.69	30.008.31	14.08	14.34	50.80	29.00	36.70	93.43	91.75	50.80	29.00	36.70	93.43	91.75	50.80	29.00	36.70	93.43	91.75
11	15.4	15.2	84	11.3	15.7	4.6	9.2	4.0	8.0	73.36	18.59	24.23	18.02	19.826.08	10.59	19.36	61.42	31.06	62.99	91.05	50.19	61.42	31.06	62.99	91.05	50.19	61.42	31.06	62.99	91.05	50.19
Av	11.9	10.9	79	11.06	14.9	4.9	9.2	4.00	7.6	63.61	24.13	43.58	20.91	21.121.61	14.41	17.45	62.22	27.17	54.15	74.14	72.96	62.22	27.17	54.15	74.14	72.96	62.22	27.17	54.15	74.14	72.96



blood calcium, and the diffusible calcium, a decrease in the calcium content of the brain, spleen, and the subcutaneous tissue

In the bile fistula dogs there was marked increase in the serum calcium, the whole blood calcium, and the diffusible calcium, an increase in the calcium content of the bone, thyroid, lung and kidney. There is a decrease in the calcium content of the subcutaneous tissue, gall bladder and adrenal and a slight decrease in the calcium content of the heart.

In each of the three jaundiced dogs and the three bile fistula dogs blood was taken each week and the serum calcium, whole blood calcium and diffusible calcium were determined. Table IV shows these results. Charts 2 and 3 show the curves of one jaundiced dog, Dog 8 and one bile fistula dog, Dog

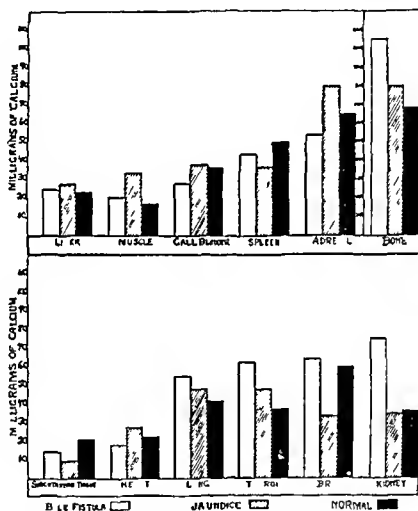


Chart 1—The tissue calcium in bile fistula, jaundiced and normal dogs

11. In the jaundiced dogs there is a steady fall in serum calcium, whole blood calcium, and diffusible calcium. In the bile fistula dogs there is a steady rise in the serum calcium, whole blood calcium, and diffusible calcium.

Feces were obtained from two normal dogs, two jaundiced dogs, and two bile fistula dogs on three consecutive days and the calcium content determined. The jaundiced dogs were jaundiced twenty-eight days and bile fistula dogs had a bile fistula seventy days. These results are summarized in Table V. The calcium content of the feces of jaundiced and normal dogs is practically the same. There is a definite decrease in the calcium content of the feces of bile fistula dogs.

On one normal dog samples of blood were taken on ten consecutive days at different times of the day, the dog being on the same diet as were all the

## CONCLUSIONS

1 There is a marked decrease in the serum calcium, the whole blood calcium and a very marked decrease in the diffusible calcium in dogs jaundiced over a period of forty days. There is also a decrease in the calcium content of the brain, spleen, and subcutaneous tissue and an increase in the calcium content of the muscle, bone, adrenal, heart, and thyroid.

2 In dogs with a bile fistula over a period of seventy-nine days, there is a marked increase in the serum calcium, whole blood calcium, and a very marked increase in the diffusible calcium. There is also a marked increase in the calcium content of the bone, an increase in the calcium content of the thyroid, lung and kidney, and a decrease in the calcium content of the feces, subcutaneous tissue, heart gall bladder and adrenal.

## REFERENCES

- <sup>1</sup>Lee, R I, and Vincent, Beth. The Relation of Calcium to the Delayed Coagulation of the Blood in Obstructive Jaundice, Arch Int Med, 1915, xvi, 59-66.
- <sup>2</sup>Grove, W R, and Vines, H W C. Calcium Deficiencies and Their Treatment by the Parathyroid, Brit Med Jour, 1922, i, 791-795.
- <sup>3</sup>Klenke, K. Condition of Serum Calcium Klin Wchnschr, 1927, vi, 791.
- <sup>4</sup>Kirk, P L, and King, C G. Calcium Distribution in the Blood, Jour Lab and Clin Med, 1926, vi, 928-935.
- <sup>5</sup>King, J H, and Stewart, H A. Effect of Injection of Bile on the Circulation, Exper Med, 1909, vi, 673-685.
- <sup>6</sup>King, J H, Bigelow, J E, and Pearce, L. Experimental Obstructive Jaundice, Exper Med, 1911, xiv, 159-178.
- <sup>7</sup>Davidson, E C, and Emerson, W C. The Influence of the Liver Upon the Toxicity of the Bile, Arch Surg, 1927, lv, 57-65.
- <sup>8</sup>Cantrow, A, Dadok, S M, and Gordon, B. Calcium Studies in Jaundice, Arch Int Med, 1927, xl, 129-139.
- <sup>9</sup>Corroy, R C, and Dennis, W. The Determination of Calcium in the Tissues, Feces, and Milk, Jour Biol Chem, 1925, lxxvi, 601-611.
- <sup>10</sup>Clark, E P, and Collip, J B. Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, Jour Biol Chem, 1925, lxxvi, 461-464.
- <sup>11</sup>Moritz, A R. The Effect of Ultra violet Irradiation on the State of Serum Calcium, Jour Biol Chem, 1925, lxxvi, 81-89.
- <sup>12</sup>Kaplanow, R, Engle, L P, and Harvey, S C. Intra abdominal Biliary Exclusion from Intestines, Cholecyst nephrostomy, Surg, Gynec and Obst, 1924, xxxix, 62-65.
- <sup>13</sup>Loeb, R F, and Nichols, E G. Influences of the Proteins on the Diffusibility of Calcium, Proc Soc Exper Med, 1925, xvii, 275.

# THE RÔLE OF COMPLEMENT IN HEALTH AND DISEASE\*

## A CLINICAL STUDY OF THE HEMOLYTIC COMPLEMENT OF HUMAN SERA

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THE scientific study of serology dates from the year 1886, when Nuttall<sup>1</sup> and Buchner<sup>2</sup> made their first observations on the role played by a substance that was inimical to living bacteria found in the majority of fresh sera. Because of the peculiar protective property displayed by this substance, it was called "Alexin."

This important observation split the newly formed school of immunology into two camps. The followers of Metchnikoff<sup>3</sup> upheld the cellular theory, while Flügge, Nuttall, Buchner, Pfeiffer,<sup>4</sup> and von Behring<sup>5</sup> were the standard bearers of the humoral school. The major part of our early knowledge concerning this protective substance we owe to the spirited controversy between these two schools, and as a result the fundamental principles of the science of serology were finally laid down by Ehrlich<sup>6</sup> and Bordet.<sup>7</sup>

Unfortunately, the study of this protective substance entered the field of academic discussion rather than practical application, and after the exposition of its nonspecificity in serologic phenomena,<sup>8, 10</sup> its importance as an "Alexin" was gradually lost sight of. Subsequent literature offers only fragmentary or indirect evidence of its role as a protective mechanism, conceived by Buchner.

The significance of alexin has been most forcibly called to our attention in the work with immune sera and therapeutic and protective vaccines. In the treatment of the chronic infective arthritides by vaccines patients with a high alexic (complementary) titer nearly always gave a favorable response, but with a low or negative one the result of treatment was doubtful or unfavorable. We also observed that in long standing debilitating diseases, the complementary titer was usually very low and in cases nearing the fatal outcome of subacute or chronic septicemia the complement was markedly subnormal if not entirely absent.

The persistent recurrence of these findings led us to believe that a careful classification of the variations of alexic titer might prove of useful prognostic value and so we undertook the present study.

Before entering into the discussion of this research, we will first define the term "complement." Alexin (Buchner), complement (Ehrlich), or addiment (Bordet) is that particular proteolytic property of active sera which reacts only in the presence of a specific antibody, a bacteriolysin, cytotoxin or proteolysin. This definition is open to criticism owing to the protean nature of the complement, but the majority of investigators of the subject are in agreement that it is a nonspecific serologic entity.

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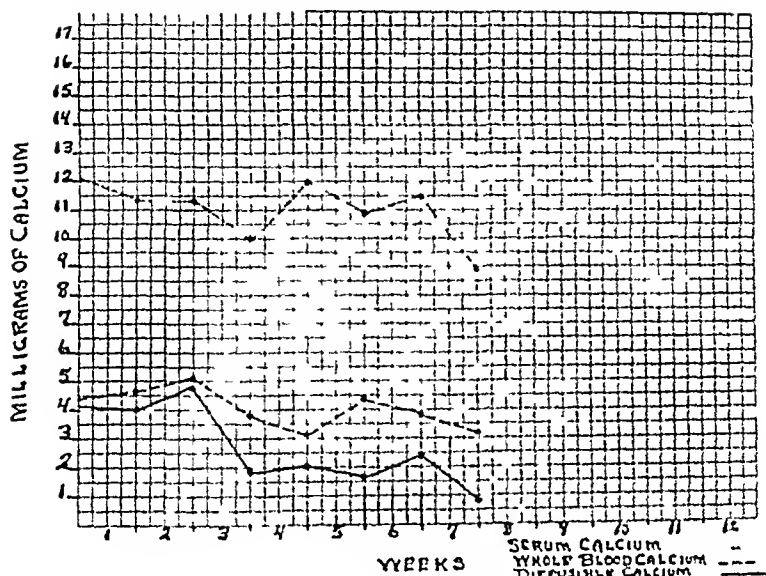


Chart 2—Curves representing the fall in serum calcium whole blood calcium and diffusible calcium in a jaundiced dog, S.

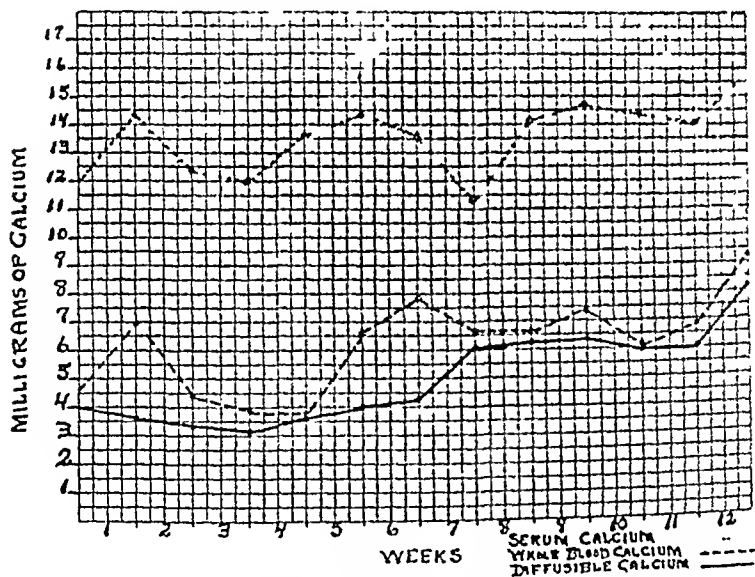


Chart 3—Curves representing the rise in serum calcium whole blood calcium and diffusible calcium in a bile fistula dog, Dog 2.

dogs used in this experiment and the serum calcium was found to vary only 0.3 of a mg.

The determination of the calcium in the bone by means of an increased or a decreased density as shown on the x-ray plate and measured by the photometer checked with the chemical determination of the bone calcium.

The calcium content of the kidney of a normal dog, the renal artery and vein of which had been clamped off, was compared with the calcium content of the opposite kidney, the renal artery and vein being intact the dog having

been bled to death and his tissues perfused. The kidney whose artery and vein had been clamped off gave a lower calcium content than the kidney that was perfused. This indicates that perfusion does not wash out any calcium from the tissues.

#### DISCUSSION

The fact that in jaundiced dogs there is a very definite decrease in the serum calcium, the whole blood calcium and especially marked decrease in the diffusible calcium would suggest that the calcium in the blood is bound by some constituent of the bile or blood. Loeb and Nichols<sup>13</sup> have shown that calcium is bound to certain of the blood proteins. Kirk and King<sup>4</sup> suggest that there may be other blood proteins that do not bind calcium and that a disturbance in the protein balance might affect the degree of diffusible calcium. The bile protein may bind the calcium of the blood also. Klink<sup>3</sup> believes that the ionized calcium is adsorbed to the proteins of the blood and splits off on dialysis passing into the outer fluid. It may very well be

TABLE V

NORMAL			JAUNDICED			BILE FISTULA		
DOG NUMBER	SAMPLE NUMBER	CALCIUM IN FECES PER 100 G M	DOG NUMBER	SAMPLE NUMBER	CALCIUM IN FECES PER 100 G M	DOG NUMBER	SAMPLE NUMBER	CALCIUM IN FECES PER 100 G M
		MG			MG			MG
1	1	29.24	-	1	187.01	10	1	2311.55
1	2	28.51			1.670	10	2	1420.12
1	3	3016.1	7		1057.61	10	3	1418.76
Average		2944.5	Average		2701.48	Average		1716.81
2	1	3044.6	8	1	4755.44	11	1	1291.81
-	2	3388.38	8	-	2458.09	11	-	1218.17
-	3	3347.0	5	3	1775.07	11	3	551.28
Average		3103.2	Average		2995.20	Average		1020.42
Average of Both Samples		306.00	Average of Both Samples		248.84	Average of Both Samples		1368.62

that the adsorption of calcium by the blood proteins is decreased in jaundice. King and Stewart<sup>5</sup> believe that the calcium of the blood is bound by the bile pigments.

When bile is shunted out of the circulation and intestines by means of a bile fistula the increase in the serum calcium, whole blood calcium and especially marked increase in the diffusible calcium would suggest that in this instance there being very little bile absorbed the calcium that was normally bound by some constituent of the bile or blood is now free in the blood stream.

There is a definite unbalance in the calcium metabolism in dogs with a bile fistula as shown by the marked decrease in the calcium of the feces and a marked increase in the bone calcium. The calcium seems to be stored in the bones.

These findings help to explain the mechanism whereby increasing the ionized or diffusible calcium of the blood by giving jaundiced patients calcium salts or parathyroid hormone will decrease the coagulation time of their blood at a time when their serum calcium is lower than normal but not nearly as low as their diffusible calcium.



## CONCLUSIONS

1 There is a marked decrease in the serum calcium, the whole blood calcium and a very marked decrease in the diffusible calcium in dogs jaundiced over a period of forty days. There is also a decrease in the calcium content of the brain, spleen, and subcutaneous tissue and an increase in the calcium content of the muscle, bone, adrenal, heart, and thyroid.

2 In dogs with a bile fistula over a period of seventy-nine days, there is a marked increase in the serum calcium, whole blood calcium, and a very marked increase in the diffusible calcium. There is also a marked increase in the calcium content of the bone, an increase in the calcium content of the thyroid, lung and kidney, and a decrease in the calcium content of the feces, subcutaneous tissue, heart, gall bladder, and adrenal.

## REFERENCES

- <sup>1</sup>Lee, R. I., and Vincent, Beth. The Relation of Calcium to the Delayed Coagulation of the Blood in Obstructive Jaundice, *Arch. Int. Med.*, 1915, **xxi**, 59-66.
- <sup>2</sup>Grove, W. R., and Vines, H. W. C. Calcium Deficiencies and Their Treatment by the Parathyroid, *Brit. Med. Jour.*, 1922, **i**, 791-795.
- <sup>3</sup>Klenke, K. Condition of Serum Calcium. *Klin. Wchnschr.*, 1927, **vi**, 791.
- <sup>4</sup>Kirk, P. L., and King, C. G. Calcium Distribution in the Blood, *JOUR. LAB. AND CLIN. MED.*, 1926, **xi**, 928-935.
- <sup>5</sup>King, J. H., and Stewart, H. A. Effect of Injection of Bile on the Circulation, *Exper. Med.*, 1909, **vi**, 673-685.
- <sup>6</sup>King, J. H., Bigelow, J. D., and Pease, L. Experimental Obstructive Jaundice, *Exper. Med.*, 1911, **xiv**, 159-178.
- <sup>7</sup>Davidson, E. C., and Emerson, W. C. The Influence of the Liver Upon the Toxicity of the Bile, *Arch. Surg.*, 1927, **xx**, 57-65.
- <sup>8</sup>Cartarow, A., Diodok, S. M., and Gordon, B. Calcium Studies in Jaundice, *Arch. Int. Med.*, 1927, **xl**, 129-139.
- <sup>9</sup>Corley, R. C., and Dennis, W. The Determination of Calcium in the Tissues, Feces, and Milk, *Jour. Biol. Chem.*, 1925, **lxvi**, 601-611.
- <sup>10</sup>Clark, E. P., and Collip, J. B. Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, *Jour. Biol. Chem.*, 1925, **lxviii**, 461-464.
- <sup>11</sup>Moritz, A. R. The Effect of Ultra-violet Irradiation on the State of Serum Calcium, *Jour. Biol. Chem.*, 1925, **lxix**, 81-89.
- <sup>12</sup>Krypinow, R., Engle, L. P., and Harvey, S. C. Intra-abdominal Biliary Exclusion from Intestines, Cholecyst nephrostomy, *Surg., Gynec. and Obst.*, 1924, **xxxix**, 62-65.
- <sup>13</sup>Loeb, R. F., and Nichols, E. G. Influences of the Proteins on the Diffusibility of Calcium, *Proc. Soc. Exper. Med.*, 1925, **xxii**, 275.

# THE RÔLE OF COMPLEMENT IN HEALTH AND DISEASE\*

## A CLINICAL STUDY OF THE HEMOLYTIC COMPLEMENT OF HUMAN SERA

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THE scientific study of serology dates from the year 1886, when Nuttall<sup>1</sup> and Buchner<sup>2</sup> made their first observations on the rôle played by a substance that was inimical to living bacteria found in the majority of fresh sera. Because of the peculiar protective property displayed by this substance, it was called "Alexin."

This important observation split the newly formed school of immunology into two camps. The followers of Metchnikoff<sup>3</sup> upheld the cellular theory, while Flügge, Nuttall, Buchner, Pfeiffer,<sup>4</sup> and von Behring<sup>5</sup> were the standard bearers of the humoral school. The major part of our early knowledge concerning this protective substance we owe to the spirited controversy between these two schools, and as a result the fundamental principles of the science of serology were finally laid down by Ehrlich<sup>6</sup> and Bordet.<sup>7</sup>

Unfortunately, the study of this protective substance entered the field of academic discussion rather than practical application, and after the exposition of its nonspecificity in serologic phenomena,<sup>8-10</sup> its importance as an "Alexin" was gradually lost sight of. Subsequent literature offers only fragmentary or indirect evidence of its rôle as a protective mechanism, conceived by Buchner.

The significance of alexin has been most forcibly called to our attention in the work with immune sera and therapeutic and protective vaccines. In the treatment of the chronic infective arthritides by vaccines, patients with a high alexin (complementary) titer nearly always gave a favorable response, but with a low or negative one the result of treatment was doubtful or unfavorable. We also observed that in long standing debilitating diseases, the complementary titer was usually very low, and in cases nearing the fatal outcome of subacute or chronic septicemia the complement was markedly subnormal if not entirely absent.

The persistent recurrence of these findings led us to believe that a careful classification of the variations of alexin titer might prove of useful prognostic value and so we undertook the present study.

Before entering into the discussion of this research, we will first define the term "complement." Alexin (Buchner), complement (Ehrlich), or addiment (Bordet) is that particular proteolytic property of active sera which reacts only in the presence of a specific antihody, a bacteriolysin, cytotoxin or proteolysin. This definition is open to criticism, owing to the protean nature of the complement, but the majority of investigators of the subject are in agreement that it is a nonspecific serologic entity.

From the Pathological Laboratories of Beth Israel Hospital New York City  
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## TECHNIC OF THE TITRATION OF THE COMPLEMENTARY VALUE

Our titration of complement is based on the hemolytic value against sheep cells which we take as an index to the bacteriolytic and proteolytic properties of the complement as well. The individual human sera are titrated in amounts varying from 0.01 to 0.10 cc. The total volume is made up to 0.50 cc. by the addition of physiologic saline. After an incubation of ten minutes, 0.50 cc. of a 2 per cent sheep cell suspension is added to each tube. The cell suspension is already sensitized with two units of antish sheep hemolysin. On further incubation for fifteen to thirty minutes, the least amount of serum capable of completely laking the added cells is taken as the complementary (hemolytic) titer.\*

Our studies of complement can be divided into two main groups. First the determination of the average combined hemolytic titer in the normal individual and under normal circumstances, in contrast to its variations in some of the common infectious diseases. Second, research bearing on the origin and nature of complement.

## THE AVERAGE HEMOLYTIC TITER OF HUMAN SERA

In a series of two thousand apparently normal cases the average hemolytic complementary titer was found to be represented by 0.04 cc. of active serum.

## THE HEMOLYTIC TITERS IN ACUTE INFECTIOUS DISEASES

The following is a list of infectious diseases investigated: typhoid fever, lobar pneumonia, acute rheumatic fever, erysipelas, osteomyelitis, acute military tuberculosis, bacterial endocarditis, and liver abscess. The appended tables are selected from many as group representatives.

*Group 1*—Acute infectious diseases running a normal course and ending in recovery without complications.

In this group we have selected one case each of four different but typical acute infections: lobar pneumonia (Fig. 1), erysipelas (Fig. 2), acute rheumatic fever (Fig. 3), and typhoid fever (Fig. 4).

The complementary curves in all cases are practically identical and may be summed up as follows:

With the onset of an acute infection, the stage at which patients are usually admitted to the hospital, there is an increase in the complementary titer. With the progress of the disease and during the fastigium, this titer stays above normal with only slight fluctuations. With the fall of the temperature, whether by crisis or lysis, the complementary titer also falls, and even though the patient be free of fever the complementary titer remains at subnormal value for about a week, at the end of which time it completes the negative phase of its cycle and mounts gradually in daily steps to reach normal value again in another week.

\*As the majority of human sera naturally contain some antish sheep hemolysin the titer as determined above is the combined hemolytic and not the pure complementary value. The effect of this native hemolysin in the amounts in which it normally occurs is of minor significance since titrations carried on with it and those performed without it by the absorption method showed only slight differences.

*Group 2*—Cases similar to *Group 1*, except for a pseudo fall of temperature, or pseudoerisis

We present two cases as representative of this group. One, a lobar pneumonia, with pseudoerisis (Fig 5), the other, a typhoid fever with a third week pseudo variation in temperature (Fig 6)

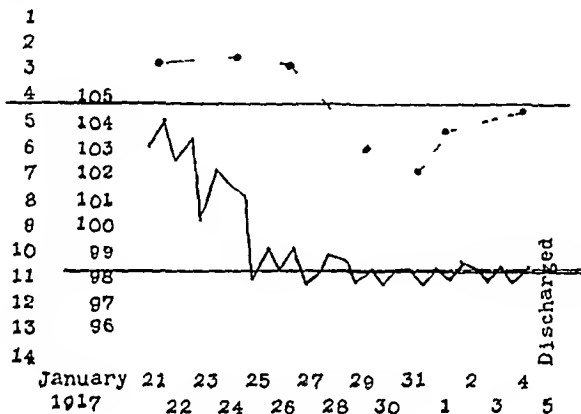


Fig 1—Case J. R. Pneumonia

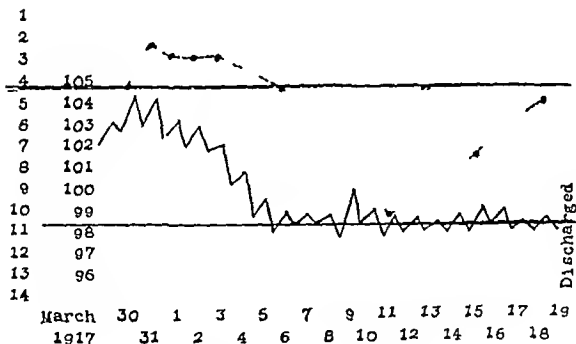
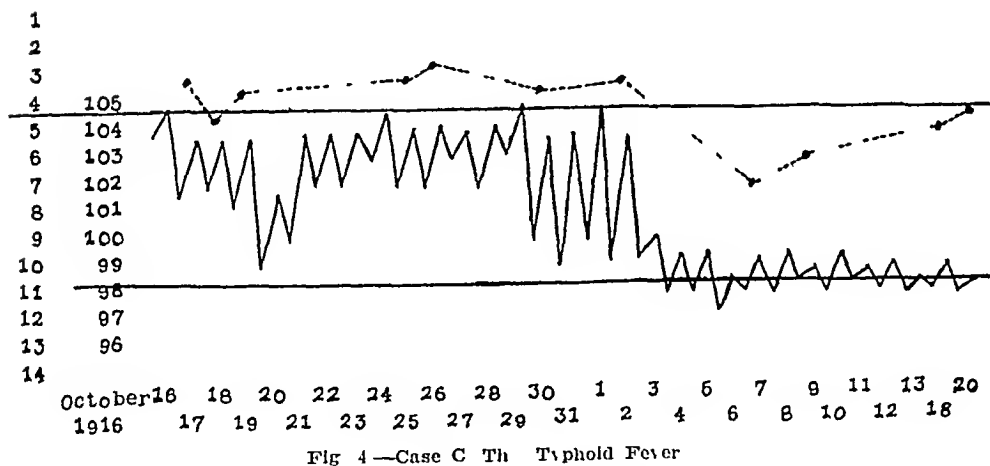
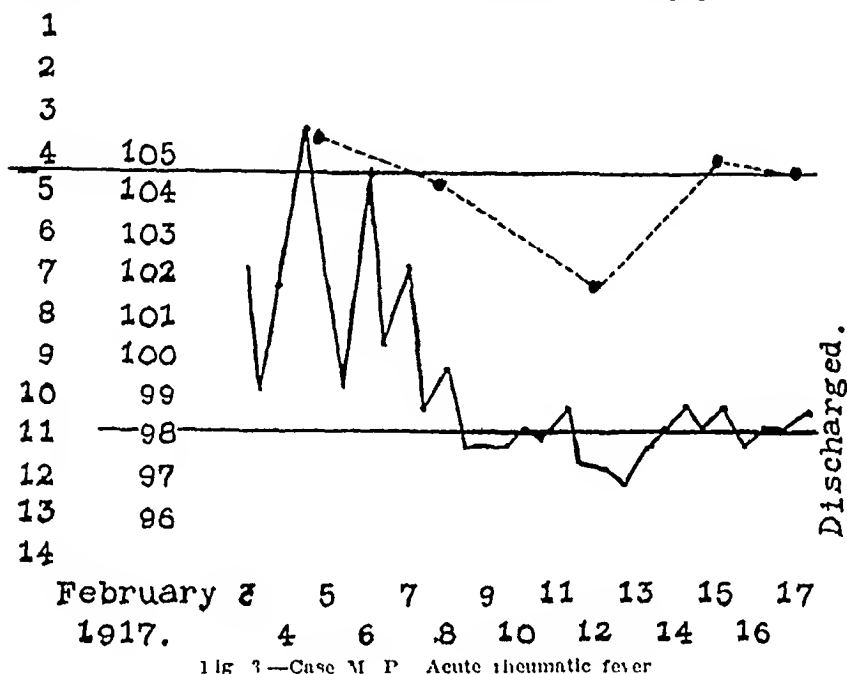


Fig 2—Case K. F. Erysipelas

Without going into the theory of the nature and production of complement which we discuss in the latter part of this paper we venture to draw the following conclusions in order to make the study of the group under discussion easier of comprehension. The close parallelism between fever and complement as represented in the first group tempts us to consider them together and as influenced by the same cause. Whatever may be the immunologic changes during a true crisis the effect on temperature and complement

is almost identical. In a pseudoe Crisis, however, the complement, unlike the temperature, departs from its normal course and with the secondary rise of temperature continues to fall until it becomes nearly twice as low as in a true crisis. But, in the absence of true complications, it gradually goes back to its normal course and eventually reaches its normal level.



### Group 3—Cases with relapse and complications

Two cases of erysipelas, one with a relapse (Fig 8), the other with relapse and complication (Fig 7), and a case of lobar pneumonia (Fig 9) are included in this group.

The cases given under this group are clinically different from Group 2, and their complementary curves deviate considerably in the following points from the course taken in the preceding group.

If the relapse occurs during the fall of complement, there is a still further fall in the titer of the latter similar to that in pseudoerisis reactions (Fig 7). If the relapse occurs following the completion of the negative phase of the complement value, then there is a sharp ascent, the subsequent course being identical with the acute infections cited under Group 1 (Fig 8). If the relapse is a severe one and its course unduly prolonged and coupled with a steady fall of complementary titer, the prognosis is very grave (Fig

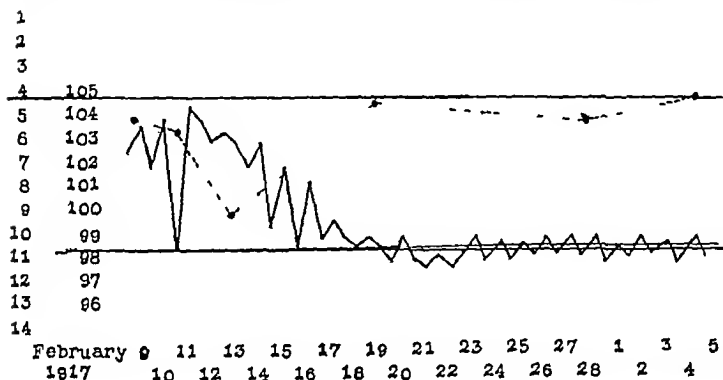


Fig 5—Case J P Pneumonia

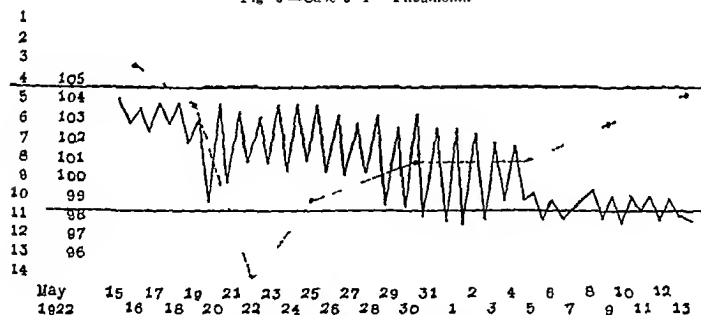


Fig 6—Case I C Typhoid Fever

9) This case could not be followed to the end but when he was removed from the hospital by his relatives he was extremely toxic

#### Group 4—Cases of acute and chronic infections terminating fatally

Three cases have been chosen to represent this group, one, a case of erysipelas (Fig 10), one of chronic rheumatic endocarditis (Fig 11), and one of chronic pulmonary tuberculosis (Fig 12)

In all of these cases the complementary titers were persistently subnormal from the time they entered the hospital. The daily fluctuations were

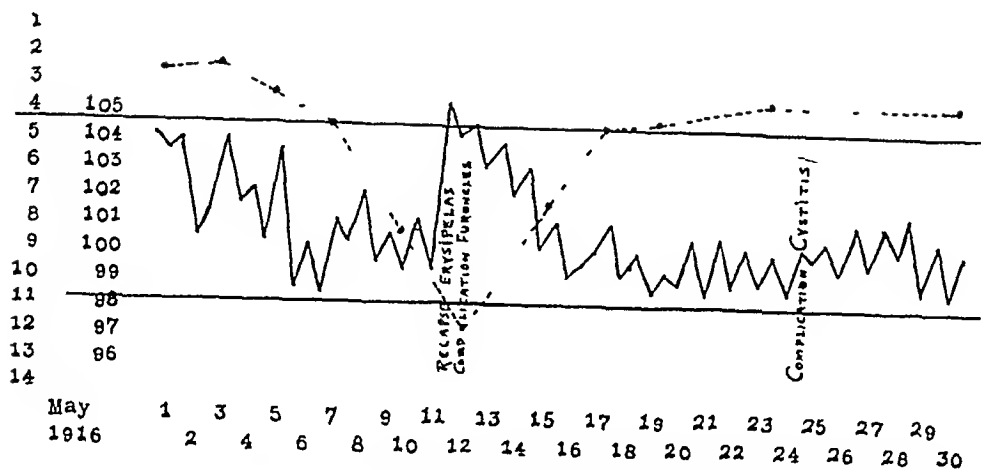


Fig 7—Case Th K Erysipelas with relapse and complicated by cystitis

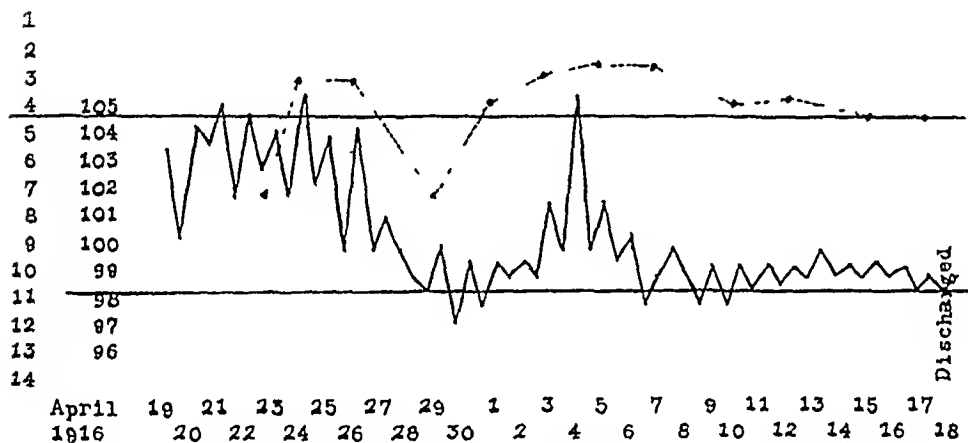


Fig 8—Case J D Erysipelas with relapse

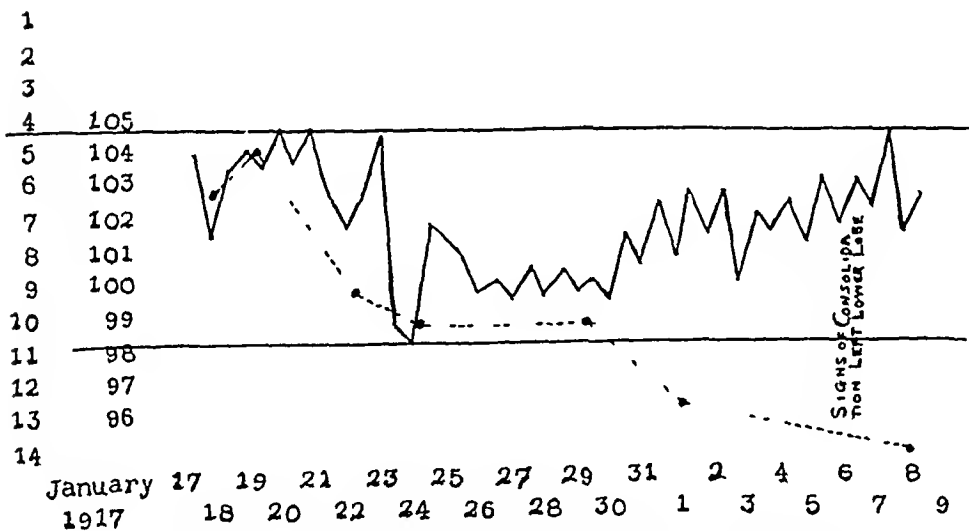


Fig 9—Case J M Lobal pneumonia with recurrence in the opposite lobe

slight, either for better or worse until there was a fall well below ordinary subnormal limits with no tendency toward improvement. In the case of B B (Fig 10) a fatal prognosis was made almost a week before it actually occurred, because of the sudden fall in complementary value at that time. Case S D (Fig 11), suffering from chronic rheumatic endocarditis, was dis-

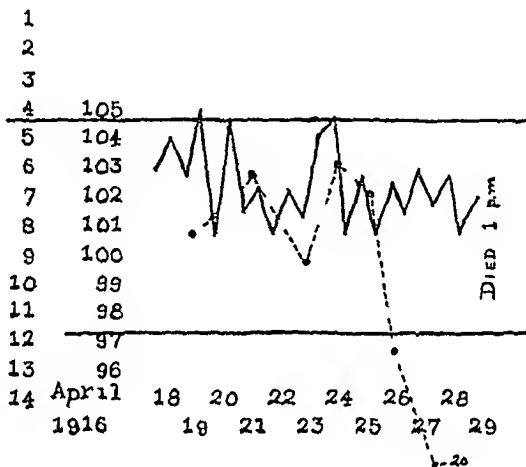


Fig 10—Case B B Erysipelas



Fig 11—Case S D Acute rheumatic endocarditis (bacterial)

charged from the hospital apparently holding his own. During his stay in the hospital the complement curve was persistently subnormal. In spite of repeatedly negative blood cultures, the prognosis on discharge, based on the complement, was unfavorable. Two months later he was readmitted with heart badly decompensated. At this time the blood culture was positive for *Streptococcus viridans*. He died shortly after the second admission. Case



A B (Fig 12), in view of the preceding discussion, needs little comment. Clinically, the patient was badly run down and had disseminated tubercular involvement of both lungs, so that the prognosis even without the complement curve was evident.

Group 5—In this group we include three cases of grave septicemia, of which Case I B (Fig 13), one of osteomyelitis with *Staphylococcus aureus* blood culture, and Case D P (Fig 15), one of deep-seated inguinal abscess.

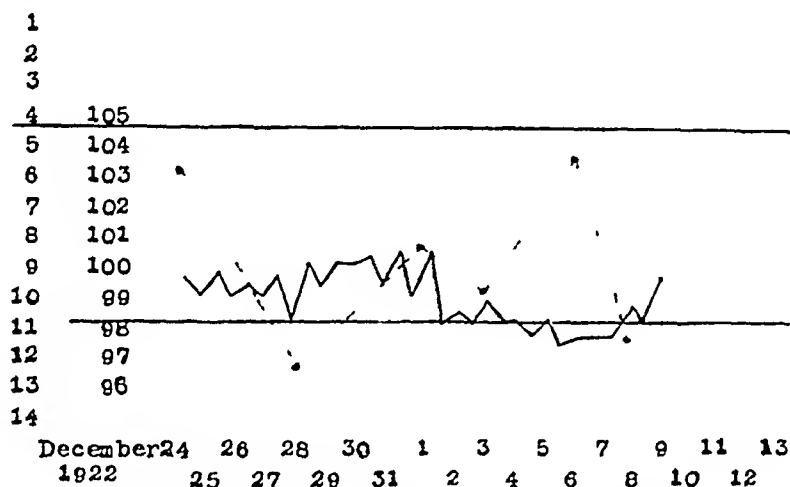


Fig 12—Case A B Chronic pulmonary tuberculosis

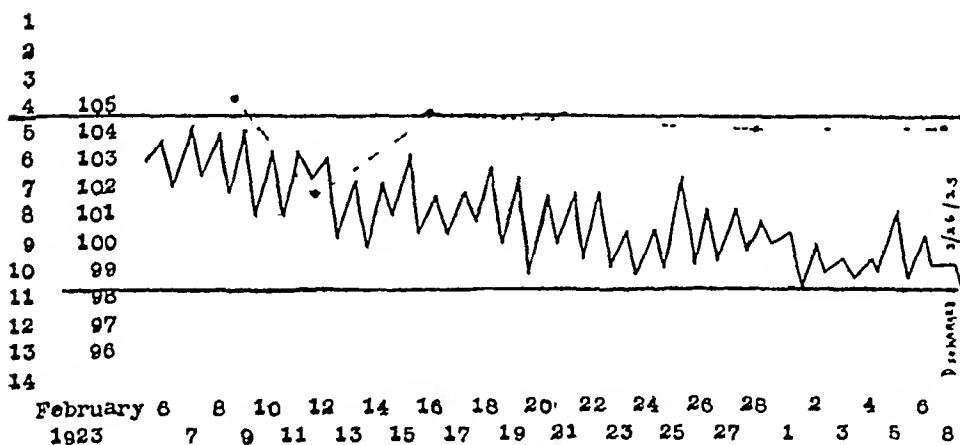


Fig 13—Case I B *Staphylococcus aureus* septicemia (osteomyelitis)

with *Streptococcus hemolyticus* blood culture, both had long septic courses, but finally recovered and were discharged with sterile blood cultures, apparently completely cured. Case G P (Fig 14) a subacute bacterial endocarditis, with persistently positive blood culture (*Streptococcus viridans*), was discharged three months after admission, on his own insistence to go home, with a fever varying between 99° and 100° during the latter part of his stay in the hospital. The subsequent course of the disease in this case unfortunately could not be followed. In view, however, of the gravity of the nature of

infection, with a complementary titer holding its own, we presume that he had an abnormally long duration for a known fatal condition

In general, even in this group of systemic infections, we find the complement undergoing the same phases as met in infectious conditions of lesser severity. The first effect of infection on the complement, after a slight fluctuation in the latter's titer, is the negative phase (the serologic shock period). The subsequent course of the disease depends on the ability of the complement to cope with the extraordinary demand

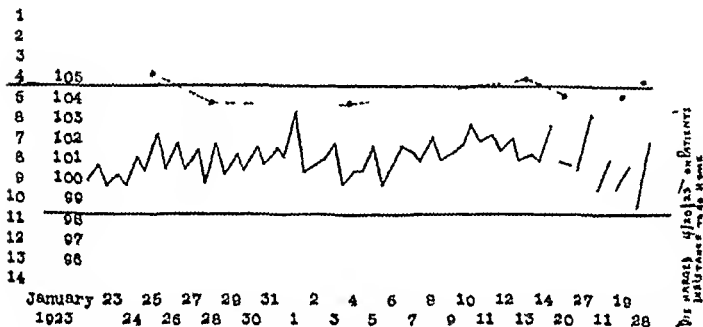


Fig 14—Case G P *Streptococcus viridans* septicemia (Subacute bacterial endocarditis)

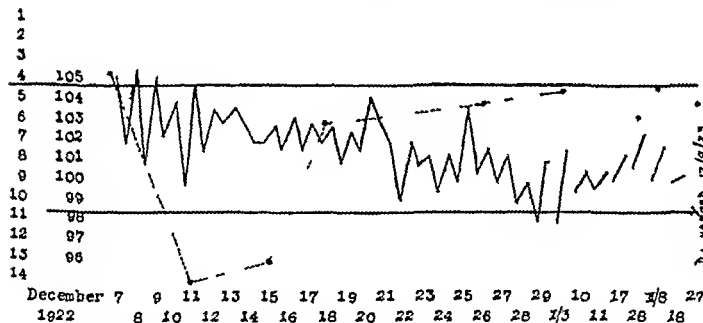


Fig 15—Case D P *Streptococcus hemolyticus* septicemia (Inguinal abscess)

#### RESEARCH BEARING ON THE ORIGIN AND NATURE OF COMPLEMENT

The question of the origin and nature of complement for the most part lies in the realm of pure speculation. The exhaustive studies of Ferrata<sup>11</sup> and Brand<sup>1</sup> disclosed its composite protein structure (albumin and globulin). It was further fractionated by Michaels and Skwirsky<sup>12</sup> into a midpiece representing the globulin fraction and an end piece, the albumin fraction.

Its origin has supposedly been traced to various internal organs to the thyroid gland by L. Fassin,<sup>14</sup> the liver by Nolf<sup>15</sup>. Serologically it is con-

sidered as a nonspecific immune element<sup>16</sup> Yet, Ehrlich and his school<sup>17, 18</sup> feel that there is a relative specificity in the bactericidal, the hemolytic, and the cytolytic complements

We do not pretend to feel that we have solved the mystery of complement, and if we venture to give certain conclusions based on our studies, we do so merely in an attempt to throw light on a phase that has hitherto been neglected We wonder if the complement is not a *catabolic product* which the living organism makes an economic use of in preventing and actually fighting disease If it be catabolite, then the complementary titer is determined by the balance of production over destruction (elimination or absorption) Complement as such is not eliminated through the regular channels

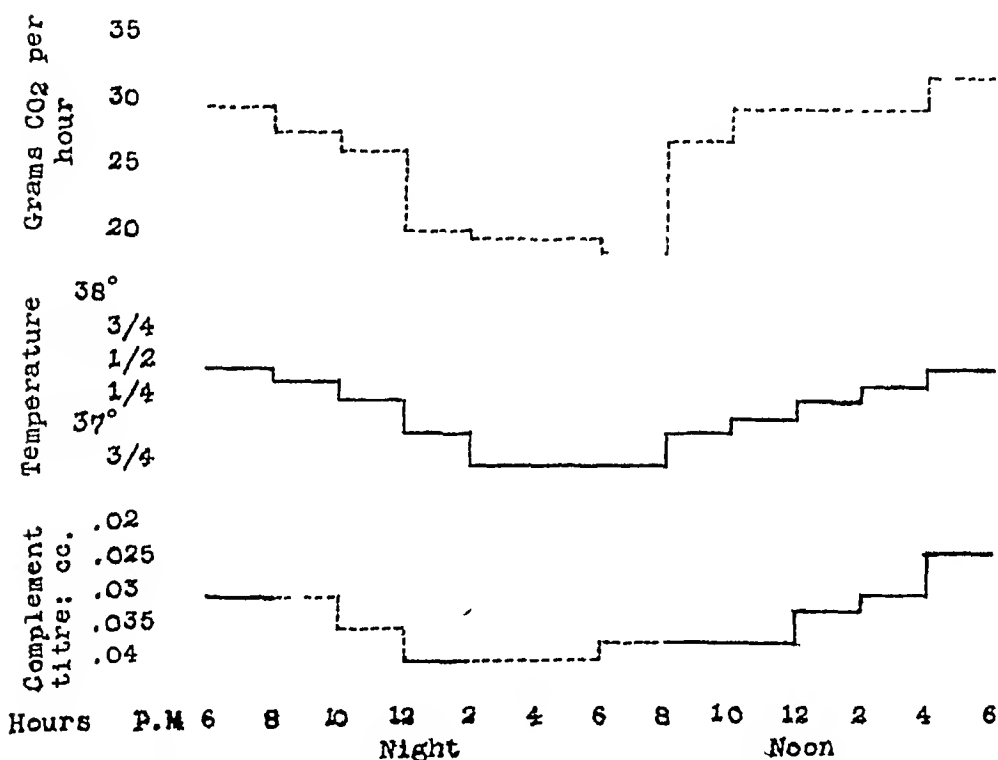


Fig. 16—Diurnal variations of metabolism, heat and complement

in urine, feces, or perspiration, consequently there must be an absorption or destruction in the physiochemical changes within the human system

Known pathologic conditions that give rise to high metabolism, such as hyperthyroidism, were also found by us to give a high complementary titer, while the reverse was found true in true hypothyroidism, cretinism, and myxedema. In acute infectious diseases, fever, taken as an index to metabolic rate, can also be used as an index to the complement. When there are departures from the above findings, such as the negative complementary phase in the early course of infections, they can be explained on the basis of an excessive consumption rather than on that of deficient production. We are already familiar with the complement neutralizing effect of protein cleavage

products, such as the peptones and albumoses, and it would seem reasonable to suppose that the consumption or inactivation of complement could be ascribed to the direct effect of proteolytic by products derived from bacteriolysis or nonspecific proteolysis under high fever<sup>10 0 1</sup>

We have collected certain experimental data in support of the catabolic theory which will be cited briefly

**Durnal Variations of the Complement**—Fig 16 gives the daily variations of the complement in relation to similar variations of body temperature and metabolism. The complement curve is the average of three findings under normal conditions. The temperature and CO<sub>2</sub> elimination curves are quoted from Lusk, *Science of Metabolism* third edition

**Effect of Exercise on Complement**—Fig 17 gives the initial complementary titer before exercise and then immediately following, and five minutes

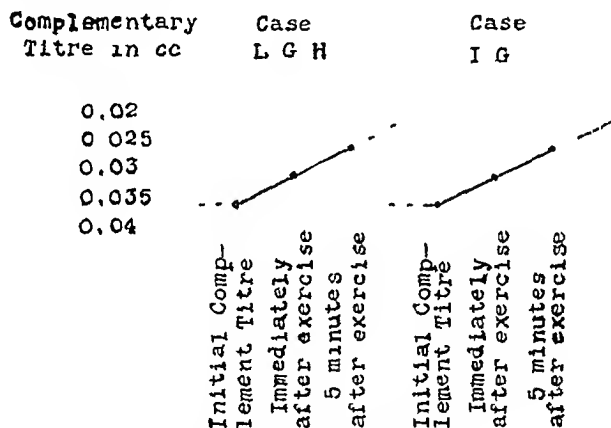


Fig 17—The effect of exercise on complement

after the strenuous exercise of climbing seventy two steps three times. The distinct rise of the complement titer is evident. Unfortunately we did not extend our observations for longer periods to determine the duration of the hypercomplementary stage for comparison as calorimetric studies of exercise usually show a lagging of the high calorie output.

**The Complementary and Metabolic Cycles in Double Tertian Malaria**—In the previous observations in acute infectious diseases no attempt was made to determine the hourly fluctuations of complement in relation to similar fluctuations in fever. In view of the fact that it is almost impossible to secure cases before the onset of temperature, such an attempt would not have answered the question we had in mind and our choice appeared to be limited either to producing a febrile reaction artificially by means of protein injections or to watching the course of a true relapse. Fortunately at this time a

case of double tertian malaria was admitted to the hospital from the dispensary service and was an ideal object for study. Two full cycles were studied and the results given in Fig 18

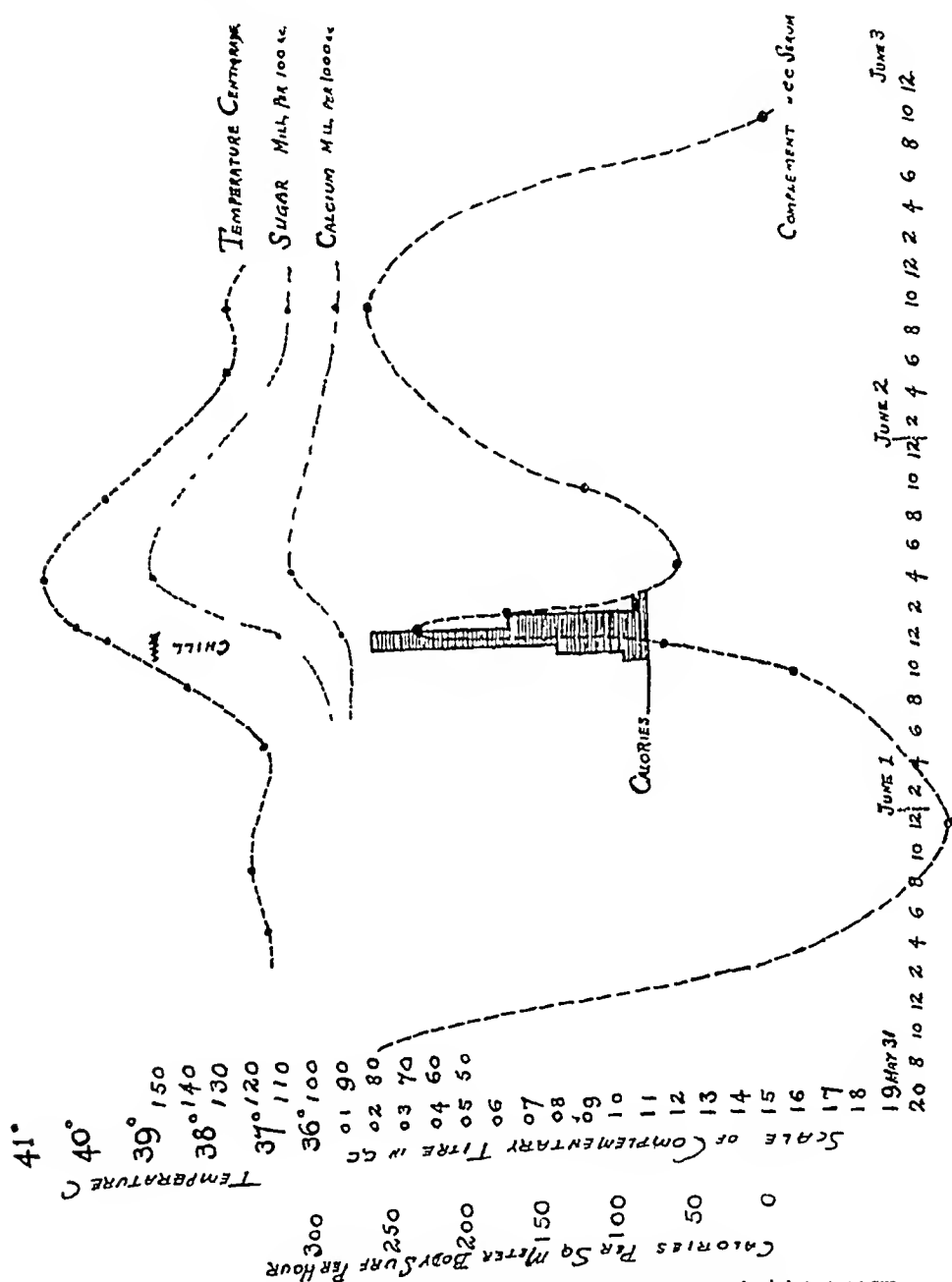


Fig 18—Hourly complementary and metabolic variations in a malarial paroxysm

As we did not have the facilities for calorimetric determinations of the metabolism during the same period we have inserted those by Barr and DuBois,<sup>22</sup> taking the period of chill for our landmark

In interpreting the complementary curve it is necessary to take into consideration, first, that with a double tertian malaria there is only one day of afebrile period and, second, that the values obtained are mean values, namely, the balance of complement production over destruction. The termination of a febrile course, such as found in malaria, gives a complement steadily falling to subnormal values, in all probability because of deficient production rather than excessive destruction. If such be the case, it would point to exhaustion of the complement producing sources and if our feeling that complement is a catabolite be correct, it would mean a relative reduction in the afebrile period, a supposition which we discovered to be true.

With the onset of the paroxysm and as we enter the chill period, there is a sharp rise in complement as well as metabolism. The metabolic rate reaching its peak about an hour earlier than the complement would point strongly to the catabolic nature of the latter.

Immediately following the chill while the temperature is still mounting to its fastigium, both the complement and the metabolic rate fall sharply. The fall of the latter becomes more gradual when basal values are reached. In the case of complement, however, the fall exceeds the regularly subnormal values until the fastigium in fever is reached, at which stage there occurs a gradual upturn in the curve and with the now steadily falling temperature reaches normal values thus bringing us to the end of the cycle.

The fall in complement immediately following the chill we ascribe not to failing production but to an increased reduction or inactivation caused by the sudden explosive discharge of proteolytic split products during the stage of chill.

#### CONCLUSIONS

1 During an infectious disease the course of the complementary, "alexie" value of the patient's serum shows considerable variation from the normal.

2 Such variations have been demonstrated by us to have prognostic significance.

3 With the onset of disease there is a steady rise in the production of the complement.

4 This stage passes rather abruptly into the *negative alexie phase*, the serologic shock period, with an abnormal fall in the complement. At this stage the fall of the alexie titer is due, in all probability, to increased complement destruction or absorption from the sudden flooding of the blood stream with proteolytic by products of known anticomplementary properties.

5 With the establishment of recovery and during convalescence the ratio between complement production and destruction is reversed, and in the course of a week the titer gradually mounts to normal values and stays there.

6 Deviation from this regular course occurs in oncoming complications, relapse or recrudescence. But a gradually mounting complement is a favorable prognostic sign. In fatal cases, the titer either falls to very low values or runs a relatively subnormal course.



# EXPERIMENTAL ACUTE MERCURIALISM\*

BY SAMUEL GOLDBLATT M.D. M.S. CINCINNATI OHIO

THE following experimental work was carried out in the attempt to throw some light upon the conflicting phenomena described in acute mercurialism

The blood chemistry of normal dogs was determined and compared with the findings in similar animals which had received lethal and sublethal doses of mercuric chloride intravenously and by mouth under various conditions

## METHOD

The animals were kept in metabolism cages and fasted for twenty four hours before the experiment. All injections were made into and the blood withdrawn from the external jugular vein. Water, and food consisting of bread, were allowed ad libitum during the course of the experiment. Blood was obtained every twenty four hours for chemical analysis unless otherwise stated on the accompanying protocols

The urea nitrogen was determined by the Van Slyke and Cullen<sup>1</sup> modification of the Marshall method. A protein free filtrate was obtained by the Haden<sup>2</sup> modification of the Folin Wu technique. From this filtrate the blood chloride content was determined as sodium chloride after the manner described by Guttler, the sugar by the method of Folin and Wu<sup>3</sup>.

Animals in which the effect of intravenous bichloride was observed received various amounts of a standard solution containing 4 grams  $\text{HgCl}_2$  to a liter of distilled water. This was injected slowly into the external jugular vein.

The influence of cholecystostomy was determined in a small group of animals. They were fasted for twenty four hours anesthetized with ether, and operated upon under aseptic technic. The common duct was exposed ligated, and resected between the ligatures. A tube was inserted into the gall bladder or the gall bladder was sewed to the abdominal wall making a permanent biliary fistula. When the animal recovered from the effect of the operation it received mercuric chloride intravenously. The chemistry of the blood was studied daily.

The time required for the absorption of a lethal dose of the poison was determined upon a series of 75 dogs. This was carried out in the following manner.

Thirty three animals were given mercuric chloride as the dry powder or in aqueous solution. The time of first emesis was noted and controlled. Ten dogs of this group were selected for a study of the blood chemical changes following the various emesis intervals.



TABLE I

PROTOCOL OF EXPERIMENTS    BLOOD CHEMISTRY    AMOUNT OF BLOOD PER 100 C C

DOG NO	WT KG	TIME DAYS	UREA N MO	CHLORIDES NaCl MO	CO <sub>2</sub> VOL.	GLUCOSE MO	N P N MG	REMARKS
1	6	1	12	480	43	100	28	
		2	10	480	37	87	21	
		3	7	440	35	85	31	
		4	9	480	37	83	27	
		5	10	520	38	90	28	
		6	11	530	35	82	31	
		7	8	540	41	75	32	
2	13	1	10	430	40			26 mg HgCl <sub>2</sub> intrav
			14	410	37			Reading sixth hour
		2	66	410	34			
		3	63	400	35			
		4	46	390	36			Dog looks well
		5	35	340	38			Dog looks well
		6	21	400	40			Dog looks well
		7	17	350	49			Dog looks well
		8	20	390	41			Dog looks well
		9	14	420	42			Dog looks well
		10	14	430	42			Dog looks well
		30	16	440	43			Had shown no symptoms
		60	14	460	45			or changes in blood chemistry since tenth day
3	15	1	10	500	45	80	26	14 mg HgCl <sub>2</sub> intrav
		2	31	420	35	64	49	
		3	94	350	21	64	137	Plasma bile stained
								Dog very sick
		4	112	250	24	71	183	Plasma bile stained
4	18							Dog very sick
		5						Dog died during night
		1	8	550	39			60 mg HgCl <sub>2</sub> intrav
		2	28	530	44			
		3	56	490	41			
		4	54	450	37			
		5	55	400	35			
		6	56	400	40	71		Refuses food
		7	60	430	37	79		
		8	68	430	34	71		Dog looks sick
		9	80	430	34	67		
		10	91	400	39	83		Appetite returns
		11	102	450	37	96		
		12	111	470	34	128		Looks very sick
		14	139	500	31	120		
		15						Dog died
5	10	1	24	450	40			40 mg HgCl <sub>2</sub> intrav
			21	460	36			Reading fourth hour
		2	56	380	25			
			59	410	23			Reading fourth hour
		3	110	400	17			Reading fourth hour
6	10		125	400	22			Dog died
		4						
		1	21	460	41			60 mg HgCl <sub>2</sub> intrav
			14	420	43			Reading fourth hour
			35	400	30			Reading eighth hour
			45	400	28			Reading thirteenth hour

TABLE I—CONT'D

DOG NO	WT KG	TIME DAYS	UREA N MG	CHLORIDES NaCl MG	CO <sub>2</sub> VOL	GLUCOSE MG	N F N MG	REMARKS
			52	400	25			Reading twenty third hour
			91	380	20			Reading thirtieth hour
								Dog died thirty sixth hour
9	14	1	21	450	40			84 mg HgCl <sub>2</sub> intrav
			28	440	36			Reading fourth hour
			42	420	21			Reading eighth hour
			38	450	10			Reading thirteenth hour
			52	400	10			Reading twenty third hour
		2						Dog died thirtieth hour
10	14	1	15	450				112 mg HgCl <sub>2</sub> intrav
			24	650				Reading first hour
			28	450				Reading sixth hour
		2	76	420				
		3	154	370				
		4	138	350				
		5	196	290				
		6						Dog died

A series of 14 animals received oral lethal doses of the bichloride dissolved in 500 to 750 cc of milk.

In a third group experiments with calcium sulphide as an immediate antidote were carried out. One gram tablets of calcium sulphide were utilized and were found to be extremely difficult of solution, even in boiling water. Aqueous solution of 7.5 grains of bichloride was administered to the animal by stomach tube and was followed immediately by a solution of 8 grains of calcium sulphide. This was carried out in 10 dogs.

In another group of 8 dogs the bichloride was dissolved in milk, administered by stomach tube and followed immediately by a solution of 8 grains of calcium sulphide.

In the last group of 10 dogs aqueous solution of mercuric chloride in lethal oral doses was administered. Gastric lavage was performed at definite intervals with a solution of 1 gram of sodium thiosulphate. This was immediately followed by a solution of 1 gram of thiosulphate which was permitted to remain in the stomach.

#### EXPERIMENTAL OBSERVATIONS

In a series of five dogs, the normal values for the blood chemistry were determined. These values are approximately the same as those described by other authors.

Chemical changes in the blood after the intravenous injection of mercuric chloride are shown in a series of twenty animals.

Four dogs received 2 mg per kg, of which three died and one recovered. The blood chemical findings in the recovered case and in one of the animals which failed to recover are shown in Chart 1.

Changes after 3 mg per kg were determined on three dogs. All the animals succumbed, having shown the characteristic increase in urea and non protein nitrogen and decrease in the chlorides, glucose and alkali reserve.

Six animals were given an injection of 4 mg per kg. Death supervened within a variable time. The typical changes in the blood chemistry, symptomatology and at necropsy were demonstrated.

Four animals received 6 mg per kg intravenously. The only way in which these animals differed from the preceding group was in the increased rapidity of the changes.

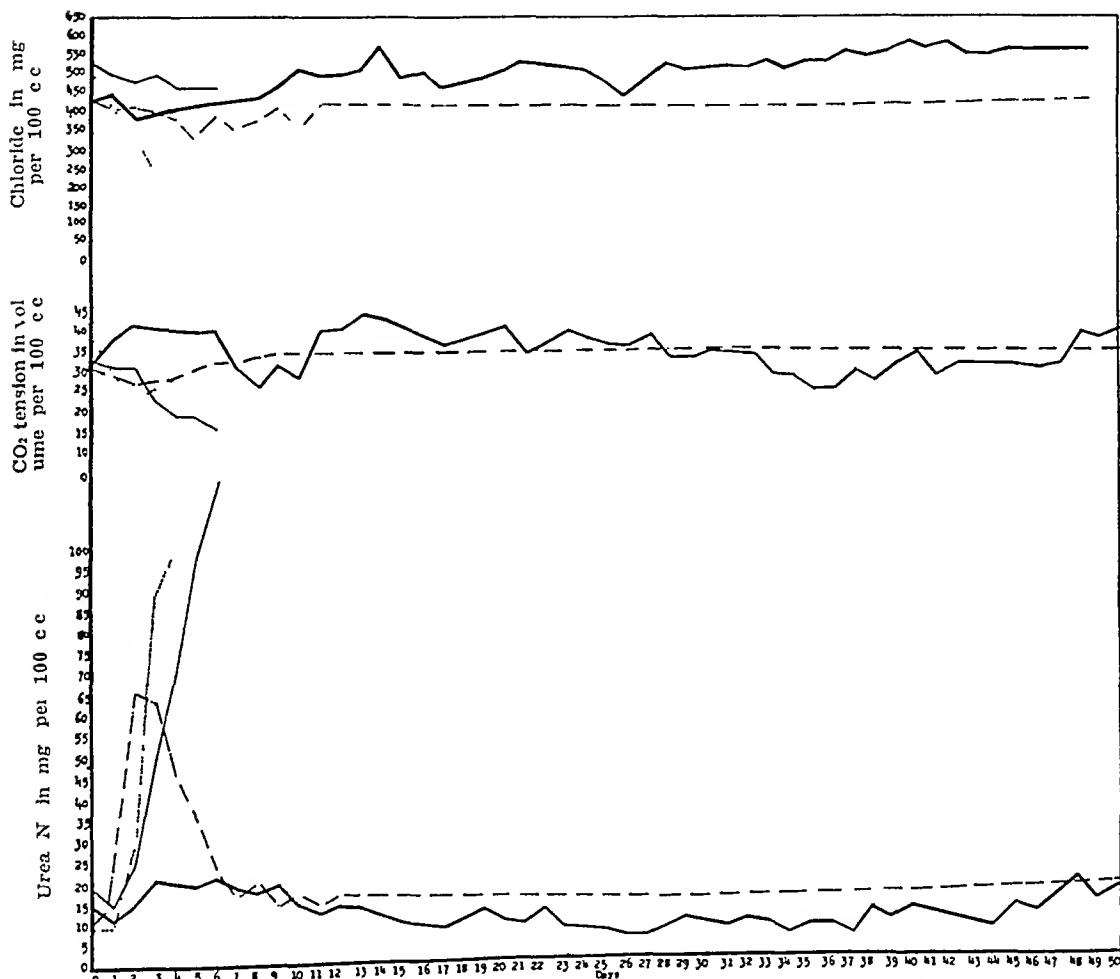


Chart 1—Blood chemistry of HgCl<sub>2</sub> intravenously 2 mg per kilogram

Normal control animal recovery — — — —

Normal control animal fatal . . . . .

Gall bladder drainage animal —————

Gall bladder drainage animal with hepatic disease —————

The last series consisted of three animals which received 8 mg per kg. Owing to the rapidity of death produced by these doses when administered intravenously, as shown in Chart 2, it was necessary to resort to the intravenous administration of sodium thiosulphate in order to keep the animals alive for a study of the blood chemistry. The changes noted under these conditions were the same as in the 4 mg group.

In Table II are shown the results of the intravenous injection of mercuric chloride in 2 to 3 mg doses per kg in five animals who have had a previous cholecystostomy. The complete inhibition of symptoms and of the characteristic changes in the blood chemistry even on repeated injections of the drug is significant. However, owing to the fact that only a small series of animals were utilized although they were carried over long periods of time, definite conclusions as to the value of this procedure as a treatment for human acute mercurialism must be held in abeyance pending further research.

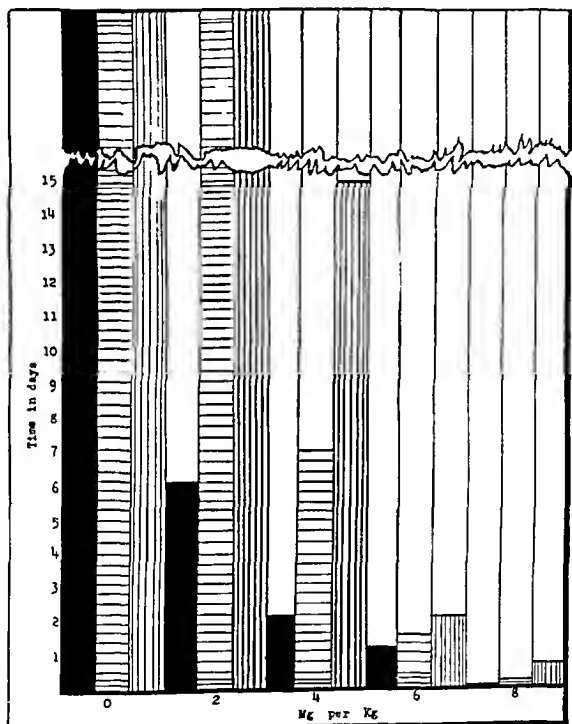


Chart —Duration of life under various doses Intravenous administration  
Minimum  Mean  Maximum

A consideration of Table III shows that dogs might die after having retained the poison for eleven minutes but did not usually die unless the drug had been retained for at least thirteen minutes. It was found that the symptoms of mercuric chloride poisoning might occur after any emesis interval (duration of time in minutes from the ingestion of the poison to first vomiting) but are not pronounced for more than one or two days unless the

TABLE II

CHEMICAL CHANGES IN BLOOD AFTER INTRAVENOUS INJECTION OF MEPCURIC CHLORIDE  
EFFECT OF CHOLECYSTOSTOMY

NO DOG	KG WT	DAYS TIME	N UREA MG	CHLORIDES NaCl MG	CO- VOL	GLUCOSE MG	N P N MG	REMARKS
3	12	1	14	440	30	45	45	
		2	10	450	36		25	24 mg intravenously
		3	15	390	40		36	Looks well
		4	20	400	39		49	Looks well
		5	20	420	38		50	Looks well
		6	19	410	38		37	Looks well
		7	20	420	38		44	Looks well
		8	19	420	29		38	Looks well
		9	16	420	24		46	Looks well
		10	18	440	29		36	Looks well
		11	15	470	26		35	Looks well
		12	12	520	38		34	Looks well
		13	14	500	38		28	Looks well
		14	13	500	42		28	Looks well
		15	11	520	41	98	32	Looks well
		16	9	590	37	76	30	Looks well
		20	8	500	34	70	28	Looks well
		21						Drainage tube out
		26	11	510	37	71	39	24 mg intravenously
		27	10	470	39	63	35	Looks well
		28	9	490	32	76	31	Looks well
		31	12	500	38	58	23	Looks well
		32	8	520	35	67	18	Looks well
		33	8	540	30	78	20	Drainage wound sealed
								Plasma contains bile
		34	8	540	34	55	21	Plasma contains bile
		35	6	530	36	75	21	Plasma contains bile
		36	6	520	31	65	23	Plasma contains bile
		37	15	500	31	47	25	Plasma contains bile
		38	10	450	32	58	22	Plasma contains bile
		40	7	500	32	62	20	Plasma contains bile
		41	9	540	26	57	23	Plasma contains bile
		42	8	520	26	38	22	Plasma contains bile
		43	6	520	23	55	20	Plasma contains bile
		47	8	530	23	58	18	Plasma contains bile
		48	8	530	28	50	31	Drainage fistula reopened
		49	6	550	24	50	20	Looks well
		50	12	530	29	45	23	Looks well
		51	10	550	32	57	25	Looks well
		52	12	550	26	57	27	Looks well
		53	11	570	30	59	24	Looks well
		54	13	560	30	66	29	Looks well
		55	10	570	30	47	34	Looks well
		56	7	600	30	59	17	Looks well
	9	57	13	580	28	45	30	18 mg intravenously
		58	10	600	30	50	27	Looks well
		59	18	570	37	65	36	Looks well
		60	14	570	36	62	28	Looks well
		61	17	580	38	62	32	Looks well
		90						Animal killed on this day
								No blood changes

interval is greater than 5 Apparently the violence of the preliminary symptoms could not be taken as an index to the prognosis in this group

There were no changes in the constituents of the blood chemistry investigated whenever the animal was permitted to vomit within five minutes However, when the emesis interval was greater than 5 the characteristic

changes of mercurial poisoning occurred in the blood chemistry. These changes were sublethal in character whenever the interval was less than 13 (Chart 3)

The use of milk in the method described above for the animals in Group 2 did not change the length of time required for a lethal dose to be absorbed. All the animals died whenever the interval exceeded 13. It could not be demonstrated that the utilization of milk in any way influenced the symptoms or complications of the toxemia.

In the dogs receiving calcium sulphide as an immediate antidote the same phenomena occurred. Regardless of whether the mercuric chloride was

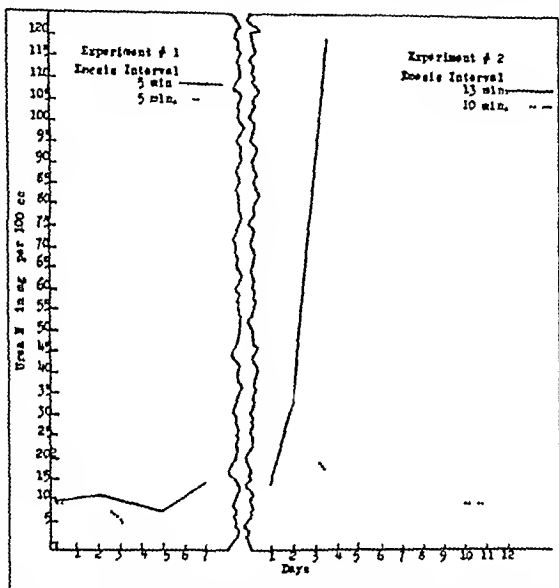


Chart 3—Blood chemistry of  $\text{HgCl}_2$  orally experimental interval twenty days

given in milk or in water solution, the prompt administration of calcium sulphide failed to save any dogs that were not permitted to vomit within the thirteen minute period.

Of the 10 animals that received gastric lavage with sodium thiosulphate those in which the procedure was delayed for more than thirteen minutes succumbed.

**Dye Absorption**—The question of the rapidity of the absorption of the blue dye which is usually used to color the commercial tablet, was investigated. The vomitus was found to be colored blue if emesis occurred within the first ten minutes. Whenever a longer interval elapsed no traces of the

TABLE III  
MORTALITY AFTER ORAL ADMINISTRATION OF MERCURIC CHLORIDE  
RELATION TO EMESIS INTERVAL

DOG NO	WT KG	DOSE MG	M L D MG	EMESIS INTERVAL	REMARKS
1	10	500	262	1 min	Dog vomited, 7.5 gr tablet placed in throat dry and washed down with 200 cc of water Vomiting and retching very marked, vomitus contained gross blood No signs after first day
2	12	500	300	2 min	Marked retching and vomiting with severe cramping noted immediately No signs after first day
3	12	300	300	5 min	Animal very sick during the first two days Marked hematemesis and diarrhea Blood chemistry showed no significant changes
4	7	225	175	10 min	No symptoms and no change in blood chemistry
5	13	500	325	13 min	Marked retching and vomiting Some hematemesis first day No symptoms thereafter
6	12	300	300	13 min	Dog very sick Died on fifth day
7	16	500	400	23 min	No definite signs of intoxication until third day Death on fourth day
8	6	225	150	30 min	Died on the second day, having shown typical findings in the blood chemistry and at necropsy
9	9	256	225	40 min	Died on fourth day Typical changes in blood chemistry

TABLE IV  
CHEMICAL CHANGES IN BLOOD AFTER ORAL ADMINISTRATION OF MERCURIC CHLORIDE  
RELATION TO EMESIS INTERVAL AMOUNT OF BLOOD PER 100 CC

DOG NO	WT KG	TIME DAYS	DOSE PER KG	EMESIS INTERVAL MIN	UREA N MG	CHLORIDES NaCl MG	CO <sub>2</sub> VOL	REMARKS
1	12	1	24 mg	5	7	500	42	Looks well
		2			14	490	42	Looks well
		3			21	530	38	Looks well
		4			17	440	36	Looks well
		5			20	500	36	Looks well
		6			18	480	39	Looks well
		7			11	450	42	Looks well
		10			13	470	45	Looks well
		35			14	480	45	Looks well
2	12	1	24 mg	13	15	580	38	Looks well
		2			33	460	32	Looks sick, refuses food
		3			65	440	22	Sick
		4			116	420	20	Very sick
		5						Died during night
3	9	1	25 mg	13	17	450	40	Looks well
		2			60	350	25	Not very active
		3			76	300	22	Very sick
		4						Died during night
4	16	1	32 mg	25	12	450	46	No signs
		3			60	400	30	
		4			84	300	22	
		5						Dog died during night
5	9	1	30 mg	75	17	400	39	
		2			60	370	28	
		3			87	290	20	
		4						Died during night

dye could be grossly detected. This held true regardless of whether the powder was given dry or in aqueous or in milk solution. The presence therefore of the blue coloration of the vomitus was taken to indicate that emesis had occurred within the first ten minutes after ingestion of the poison, and consequently permitted a very good prognosis with reference to fatality.

*Influence of Previous Feeding*—Animals which recovered were subjected to experiments to determine the effect of previous feeding or fasting. It was found that the animal which had previously been fed, vomited spontaneously earlier than the animal which had been fasted for a twenty four hour period. When the emesis interval was controlled, the presence of food in the stomach did not prevent the absorption of a lethal dose within the thirteen minute period.

*Blood Coagulation*—It was incidentally noted during the study of the blood chemistry that blood coagulation occurred much more rapidly during the acute toxemia than before or after it and that the amount of potassium oxalate necessary to prevent clotting frequently had to be double or triple that which would inhibit it in normal dogs.

*Autopsy Findings*—Necropsies were performed upon all animals. The finding of marked emaciation and immediate rigor mortis was characteristic. Hyperthermia was noted in many animals immediately after death. The evidences of salivation and bloody diarrhea were frequently seen.

The brain was yellowish gray in color, suggesting fatty degeneration. The cortical vessels were dilated and the vessels of the pia arachnoid were congested. The heart was practically always dilated and usually filled with postmortem clots. The lungs were collapsed, showing marked congestion of the parenchyma and occasionally very marked subpleural hemorrhages. When death occurred immediately following the administration of the drug either by mouth or by vein, intense edema of the lungs was the most characteristic finding. The liver was enlarged, congested, and fatty. Animals which lived for a long time (fourteen days) showed a typical picture of acute yellow atrophy. The spleen was slightly hyperemic but otherwise grossly not affected. The kidneys were large, pale, purple soft, and congested in these animals. When immediate death took place kidney changes were manifested by large purple swelling. On section the glomeruli were not visible. The medulla was intensely congested and a characteristic pale yellow line ran through the center of the cortex. Examination of the gastrointestinal tract showed the esophagus lined with a brownish mucoid exudate, the stomach contracted with the rugae prominent and presented small petechial hemorrhages. The duodenum was hemorrhagic and ulcerated. The ileum and jejunum were usually clear although the colon was invariably intensely hemorrhagic with marked sloughing of the mucosa.

#### CONCLUSIONS

- 1 The blood chemistry of normal dogs was found to be within the limits described by other authors.



2 Blood chemical changes following the intravenous or oral administration of  $\text{HgCl}_2$  were characteristic

- a Marked increase in the urea nitrogen
- b Parallel increase in the nonprotein nitrogen
- c Decrease in the chloride content
- d Decrease in the sugar content
- e Decrease in the alkali reserve

3 The minimum lethal dose established by Sansum for  $\text{HgCl}_2$  administered intravenously as well as the anemic dose of this author is confirmed

4 The mortality following the administration of bichloride orally varied directly as the emesis interval

5 Blood chemical changes following the oral administration of  $\text{HgCl}_2$  are proportional to the emesis interval

6 The mortality following the oral administration of  $\text{HgCl}_2$  in milk varied directly as the emesis interval

7 The use of calcium sulphide as an immediate antidote did not influence the rate of absorption of a lethal dose of the poison

8 The antidotal value of sodium thiosulphate when used as a gastric lavage could not be demonstrated if utilized after a thirteen minute interval

9 The time required for the absorption of a lethal dose of mercuric chloride dry, in aqueous solution or in milk was approximately thirteen minutes

10 The time required for the absorption of the blue dye with which the tablets are colored was approximately ten minutes

11 There was a marked lowering of the coagulation and bleeding time during acute mercurial toxemia

12 Chemical changes in the blood and the symptoms presented by the animal after the intravenous administration of mercuric chloride were prevented by cholecystostomy

13 Sudden death following the intravenous or oral administration of large doses of the drug indicated involvement of the nervous system (convulsion), or of the cardiovascular system (shock), or of the pulmonary system (edema of the lungs)

14 The gastro-enteric system, the liver and kidneys were involved almost immediately but death from these causes was usually delayed for several days

#### REFERENCES

- <sup>1</sup>Van Slyke, D, and Cullen Jour Am Med Assn, 1914, lxi, 1588
- <sup>2</sup>Haden, R L Jour Biol Chem, 1923, lvi, 469
- <sup>3</sup>Gettler, A O Jour Am Med Assn, 1921, lxxvii, 1652
- <sup>4</sup>Folin and Wu Jour Biol Chem, 1920, xli, 367

# LABORATORY METHODS

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## THE USE OF DOGS IN THE ASSAY OF DIGITALIS\*

### A COMPARISON WITH THE RESULTS OBTAINED ON CATS

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THE important place in therapeutics occupied by digitalis renders it extremely desirable that at least approximate uniformity be secured in the potency of commercial preparations of the drug. The recent report of van Wijngaarden<sup>6</sup> furnished additional evidence as to the unreliability of chemical methods for assay of digitalis and supported the view that attention must still be directed toward physiologic tests in the attempt to standardize the preparations. For some twelve years, the cat method of Hatcher and Brody<sup>2</sup> has been used in this laboratory, an analysis of the results that have been obtained with this method has been published previously.<sup>1</sup> Here, it suffices to state that we are convinced that the method is sufficiently accurate for practical purposes, provided a sufficient number of cats is used in each assay. Unfortunately, it has been our experience that it is difficult to secure cats in large enough numbers at certain times, that our experience is not unique is evident from the statements of other workers. Knapp Lenz has recently proposed<sup>4</sup> the substitution of guinea pigs, and, employing a technic similar to that proposed by Hatcher and Brody for cats. Knapp Lenz has secured quite satisfactory results by slow intravenous injection of the digitalis solution into the jugular veins of the pigs. Some years ago, Rowe suggested<sup>5</sup> the use of dogs for digitalis assay, but considered that these animals were unsatisfactory, because the toxicity of different samples of digitalis and strophanthus did not show the same ratio on cats and on dogs. With us, for certain reasons, dogs would be preferable to either cats or guinea pigs, dogs can be secured in large numbers, the necessary dissection is simple, and they are cheaper than guinea pigs. In view of the experience as to the large number of cats required to attain approximate accuracy in the assay of digitalis, we suspected that Rowe's conclusions were not justified, because of his failure to use a sufficient number of animals, either cats or dogs, in most of his assays. Having recently tested a sample of tincture of digitalis on a total of 81 cats,<sup>2</sup> it seemed that opportunity was offered of obtaining information of interest by testing the same tincture on a large number of dogs.

In carrying out the assay of digitalis on dogs, the animals were first given 10 mg of morphine sulphate per kilogram subcutaneously and were then etherized for the operative procedures. Because of ease in handling the

animals and for the sake of economy in the use of digitalis, small dogs were used in most cases, many of these were full grown, some were quite immature. The tincture (W-26, percolated in 1926), diluted with nine parts of isotonic salt solution, was injected into the femoral vein at the approximate rate of 3 c.c. of the dilution every two and one-half minutes. Artificial respiration was not used, because we feel that this rather lessens the sharpness of the end-point and has no compensating advantage. On the appearance of marked cardiac weakness, the rate of digitalis injection was materially reduced.

A total of 76 dogs received injections of the tincture W-26 in the manner described. It was planned to use groups of six animals each on different days, on two occasions, however, a dog was accidentally killed with ether, consequently, there are eleven groups, each with six, and two groups, each with five individuals. In Table I are given the results of these tests.

The average lethal dose from all dogs in the series was 140.8 mg. per kilogram, the same tincture tested on 81 cats was found to have an average lethal dose of 79.3 mg. per kilogram. The smallest amount of digitalis required to cause the death of a dog was 76.3, the largest, 241 mg. per kilogram, differences greater than these were encountered with cats, where the extremes were 45.3 and 123.2 mg. per kilogram. Of the 76 dogs, the lethal dose for 38 fell below the average and for 38 was above the average.

Considering the averages for the separate groups, the results appear definitely less satisfactory than those obtained on cats. Thus, of the 13 dog groups, the averages of only five fell within 10 per cent of the general average, while thirteen of the fourteen cat groups came within this limit. The high group average for dogs, 165.6 mg. per kilogram, was 17.6 per cent above, the low group average for dogs, 121.1 mg. per kilogram, was 14.0 per cent below the general average, as compared with 16.2 per cent above and 9.5 per cent below the general average for corresponding cat groups. In almost every respect, it seems that dogs are less satisfactory than cats for the assay of digitalis.

Several factors may have been responsible for the comparatively poor showing secured by the use of dogs in testing this sample of digitalis. As already mentioned, the size of the animals used was not uniform. The average weight of the dogs was 5.85 kilograms, the extremes were 2.2 and 16.0 kilograms. The average lethal dose for 42 dogs weighing less than 5.85 kilograms was 142.8 mg. per kilogram, that for 34 dogs weighing more than 5.85 kilograms was 135.6 mg. per kilogram. This suggests the possibility that the weight of dogs may have an influence in causing variations in susceptibility to digitalis greater than was found to be the case with cats.<sup>2</sup>

In 11 instances, note was made that the animal used was a puppy, the average lethal dose for these 11 puppies was 148.0 mg. per kilogram. It might be inferred from this that young animals have a higher resistance to digitalis than older dogs possess, however, the limited number of observations scarcely justifies this.

Although our experience indicates that dogs are distinctly less satisfactory than cats for the assay of digitalis, it is obvious that the employment of a sufficiently large number of dogs for each assay will afford results not far

TABLE I  
ASSAY OF TR. W 26 ON DOGS

DATE	DOSE OF DIGITALIS MG x KG	AVERAGE
Sept 29	133.2	133.3
	142.0	
	141.9	
	122.8	
	133.3	
Oct 4	114.4	146.2
	150.2	
	97.3	
	122.7	
	170.0	
Oct 5	241.0	136.4
	144.4	
	150.0	
	157.0	
	110.6	
Oct 10	100.0	137.9
	146.4	
	146.2	
	122.2	
	183.1	
Oct 12	133.3	126.4
	124.4	
	118.5	
	126.0	
	133.0	
Oct 25	125.8	142.7
	160.0	
	101.5	
	112.4	
	141.5	
Oct 26	156.9	122.9
	146.1	
	150.8	
	140.5	
	120.6	
Oct 31	160.6	158.2
	116.4	
	76.3	
	128.8	
	127.4	
Nov 1	194.1	121.1
	133.3	
	124.0	
	162.6	
	142.4	
Nov 8	192.8	143.6
	121.5	
	142.7	
	118.4	
	105.8	
Nov 22	113.4	165.6
	125.1	
	113.3	
	160.0	
	162.0	
	143.0	
	150.0	
	133.3	
	142.2	
	194.7	
	145.0	
	149.3	

TABLE I—CONT'D

DATE	DOSE OF DIGITALIS		AVERAGE
	MG	KG	
Dec 6	151.3		135.9
	211.2		
	142.6		
	150.0		
	113.1		
	108.2		
Dec 14	132.4		149.6
	109.3		
	163.1		
	135.1		
	151.6		
	137.2		
	140.6		
Average of total		140.8	

from the probable potency of the preparation tested. If, however, it is true that the relative toxicity of different samples of digitalis is not the same on cats and on dogs, doubt would be cast on both methods of testing digitalis. The lethal dose of Tr W-26 for dogs is 1.77 greater than that for cats, by reference to figures obtained from previous tests, it has been possible to estimate the relative toxicity of other digitalis preparations on dogs and on cats, the results of such estimation are given in Table II.

TABLE II

RATIO OF TOXICITY OF QUABAIN AND OF TINCTURES OF DIGITALIS AS DETERMINED ON CATS AND ON DOGS

PREPARATION	LETHAL DOSE IN MG \ KG		RATIO
	DOGS	CATS	
Ouabain	0.178	0.0991	1.80
Tr W 26	(8)*	(27)*	1.78
	140.8	79.3	
	(76)*	(81)*	
Tr 33	126.8	76.5	1.66
	(6)*	(12)*	
Tr 36	74.3	41.3	1.80
	(5)*	(12)*	
Tr 51	145.3	83.1	1.75
	(7)*	(14)*	

\*Number of animals used

It is obvious from the figures in Table II that the ratio of toxicity for the different samples of digitalis bodies is so near the same, although a comparatively smaller number of animals was used in some cases. It would appear that the lethal dose of a digitalis body is consistently higher for dogs, and, so far as reliance may be placed on this limited number of observations, it is possible to translate the results of an assay of digitalis on dogs to the more familiar "cat units" by dividing the lethal dose for the dogs by a figure around 1.75.

It may be concluded that, so far as accuracy is concerned, cats are superior to dogs for the assay of digitalis. Nevertheless, a fairly satisfactory assay of digitalis may be carried out by the utilization of from six to ten dogs in each test. So far as the limited number of samples compared justifies infer-

ences, it may be assumed that the relative toxicity of different digitalis preparations is the same on dogs and on cats \*

## REFERENCES

- <sup>1</sup>Haskell and Courtney *Am Jour Med Sc*, 1924 *clviii* 816  
 Haskell C C In press  
<sup>2</sup>Hatchor and Brody *Am Jour Pharmacy* 1910 *lxxii* 360  
<sup>3</sup>Knafl Lenz *Jour Pharmacol and Exper Therap*, 1926 *xxix* 40,  
<sup>4</sup>Rowe, L W *Jour Am Pharmacol Assn* 1919 *viii* 343  
<sup>5</sup>Vaa Wijngaarden *Arch f Exper Path und Pharmacol* 1921 *cxvii* 135

MEDICAL COLLEGE OF VIRGINIA

## THE DETERMINATION OF AMMONIA BY AERATION†

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IN THE determination of ammonia by the aeration method of Van Slyke and Cullen (1916) one meets with the inherent difficulties of the use of very dilute acids and alkalis namely, the satisfactory standardization of these reagents, their keeping qualities and the judgment of the end point of the titration. In the course of some investigations requiring the aeration method for the determination of ammonia we have introduced two procedures, one of which eliminates the use of the standard alkali and the other increases the accuracy of the titration. Our method is as follows

The apparatus of Van Slyke and Cullen is set up exactly as described by them, excepting that in the receiving tube 40 cc of a 4.5 per cent boric acid solution measured approximately, is used instead of 25 cc of N/50 hydrochloric or sulphuric acid. The aeration is then carried out at an initial rate of between three and four liters of air per minute for five minutes and then at a rate of twelve liters for twenty five minutes. The ammonia absorbed by the boric acid is titrated with N/56 hydrochloric acid using methyl orange or bromophenol blue as the indicator, the end point being accurately determined by comparison with the following arrangement of tubes providing a standard which is readily reproducible in shade and intensity of color. The titration is conducted in a four holed comparator block. One side of the block has two Van Slyke and Cullen aeration tubes containing 80 cc of water. With methyl orange as an indicator one of these tubes contains 0.5 cc of 0.04 per cent methyl orange and a drop of concentrated hydrochloric acid; the other tube contains 15 cc of the methyl orange solution and one drop of 10 per cent sodium hydroxide. If bromophenol blue is used each tube contains 80 cc of water, 1 cc of 0.04 per cent bromophenol blue, and is respectively acid and alkaline as above. The other side of the block holds an aeration tube of distilled water and the experimental tube with 2 cc of the indicator solution. As the end of the titration is approached the experimental

Tests carried out since the completion of the experiments described above suggest that there is apparently a seasonal variation in the resistance of dogs toward ouabain. Certainly our statement that the fatal dose of this substance for dogs is 0.178 mg per kilogram requires modification during the past summer it has been found to be 0.139 mg per kilogram. This would give an entirely different ratio for ouabain and for digitalis on cats and on dogs.

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tube is diluted to approximately 80 cc with water neutral to, but not containing, the indicator in use. The tubes in the comparator block are illuminated by the light from an ordinary 100 watt lamp reflected from a sheet of white paper.

## DISCUSSION

Winkler (1913) proposed the use of boric acid to receive the ammonia in the determination of nitrogen by the Kjeldahl method and the direct titration of the ammonia. Adler (1916) and Scales and Harrison (1919) found the method entirely satisfactory. The procedure effects a saving in the time of measuring the solution for the ammonia absorption, reduces the number of readings of burettes from two to one, eliminates the use of the comparatively unstable standard alkali solution, provides a large capacity for ammonia absorption, and conserves the standard acid solution employed, as the amount used is equivalent to the nitrogen involved. Kober and Graves (1913) attempted to apply boric acid to the fixation of ammonia in aeration. They reported that ammonia escaped from boric acid and could be detected in a second absorption tube by nesslerization. We have conducted a similar experiment and compared the ammonia holding efficiency of 40 cc of 4.5 per cent boric acid with that of 25 cc of N/14 hydrochloric acid. The aeration rates as given above were employed. Twenty-five cc of N/56 hydrochloric acid were placed in a second absorption tube. At the close of the aeration the boric acid tubes were titrated with N/14 hydrochloric acid and the second absorption tubes of each series were transferred to 100 cc flasks, treated with 10 cc of Nessler's solution, diluted to the mark, and compared with standards. The results of a typical experiment as recorded in Tables I and II show that *in aeration a 4.5 per cent solution of boric acid is apparently just as effective in absorbing and retaining ammonia as N/14 hydrochloric acid*. The experiment also shows the capacity of 40 cc of boric acid to be far in excess of the amounts of ammonia commonly determined by aeration.

TABLE I  
AERATION INTO 40 CC OF 4.5 PER CENT BORIC ACID

NUMBER OF SAMPLE	NH <sub>3</sub> -N PRESENT MG	TITRATION NH <sub>3</sub> -N MG	NESSLERIZATION NH <sub>3</sub> -N MG
1 A	5.00	5.00	less than 0.010
2 A	10.00	10.00	less than 0.025
3 A	15.00	15.00	less than 0.025
4 A	20.00	20.00	0.030
5 A	25.00	25.00	0.050

TABLE II  
AERATION INTO 25 CC N/14 HYDROCHLORIC ACID

NUMBER OF SAMPLE	NH <sub>3</sub> -N PRESENT MG	NESSLERIZATION NH <sub>3</sub> -N MG
1 B	5.00	less than 0.010
2 B	10.00	less than 0.025, and less than 2 A
3 B	15.00	less than 0.025, equivalent to 2 B
4 B	20.00	less than 0.050, but greater than 4 A
5 B	25.00	0.050

The precision of the method is revealed by the following experiment. Various dilutions of pure ammonium sulphate were made by one of us and the series of "unknowns" analyzed by the other. The results appear in Table III.

TABLE III

NUMBER OF SAMPLE	NH <sub>3</sub> -N PRESENT MG	BROMOPHENOL BLUE	METHYL ORANGE
		NH <sub>3</sub> -N FOUND MG	NH <sub>3</sub> -N FOUND MG
1	3.24	3.16	3.18
2	5.32	5.21	5.18
3	0.90	0.87	0.90
4	4.98	4.94	4.95
5	6.31	6.30	6.30
6	1.49	1.49	1.51

The use of bicolor standards in a comparator block for the determination of titration curves was suggested by Gillespie (1920). His method, however, differs from ours in that he uses only a part of his solution for comparison and that he was interested in the actual  $P_H$  of his solutions. We have the entire solution in the comparator block and make use of the bicolor system only to fix an arbitrary, readily reproducible end point, not necessarily of known  $P_H$ .

We have found that caprylic alcohol interferes with nesslerization for the determination of the traces of ammonia carried through boric or hydrochloric acid. By substituting a drop of phenyl ether (Lee, 1917) in each tube we have entirely eliminated the interference and have found it to be just as effective an antifoamer as caprylic alcohol. Phenyl ether is not dispersed by aeration or stirring as readily as caprylic alcohol and so does not interfere with observing the true color of the indicator due to any cloudiness of the solution. It can be obtained for one tenth the cost of the alcohol.

For convenience the entire apparatus with the exception of the light source is arranged on a single iron stand with the comparator block raised to eye level. Stirring is effected by means of a footed glass rod operated by a string pulled through a short, inverted U tube supported above the comparator block.

It is necessary in our procedure to control the concentration of the indicator. An alternative to the method of dilution as given above is to titrate with N/56 hydrochloric acid containing 10 mg of methyl orange or bromophenol blue per liter. Using this method we use the bicolor standards as given above but only 1 cc of the indicator solutions in the experimental tube. Any wash water added should be neutral to the indicator in use and contain 10 mg of methyl orange or bromophenol blue per liter.

## REFERENCES

- Adler, L. Z. ges. Brauw. 1916 xxxix, 161. Chem. Abstr. 1917, xi, 3371.  
 Gillespie, L. J. Jour. Am. Chem. Soc. 1920 xlii, 742.  
 Kober, P. A., and Graves, S. S. Jour. Am. Chem. Soc. 1910, xxxv, 1594.  
 Lee, O. I. St. Luke's Hosp. Med. and Surg. Rept. 1917 iv, Chem. Abstr., 1917 xi, 1808.  
 Seales, F. M., and Harrison, A. P. Jour. Ind. and Eng. Chem., 1920, xii, 309.  
 Van Slyke, D. D., and Cullen, G. E. Jour. Biol. Chem. 1916, xxiv, 117.  
 Winkler, L. W. Z. anorg. Chem., 1913, xvi, 231.



# THE CLINICAL VALUE OF THE SALIVARY UREA INDEX\*

BY CLAIR L. STEALY, M D, SAN DIEGO, CALIF

SINCE determinations of blood urea nitrogen and creatinine are very important factors in the estimation of renal impairment, an easily applied and accurate method of estimation which could be used routinely or as a simple indicator to sift out cases of renal impairment with retention for more careful study would be of great value in the everyday practice of medicine. Our present methods are invaluable but the training and equipment necessary prohibit their use by all except the larger institutions and laboratories. Also, while urinary findings are simple indicators of kidney impairment, we do not like to subject every patient whose urine contains albumin or casts, or shows a fixation of specific gravity, to these more complicated tests. Therefore, when Hench and Aldrich<sup>1</sup> published their findings of a simple method for using the salivary urea nitrogen determination as an index to the blood urea nitrogen, it was seized upon as at least an indicator which would sift out cases of renal impairment with retention for more detailed study.

Marshall and Davis<sup>2</sup> and Myers and Fine<sup>3</sup> have demonstrated that urea nitrogen is diffusible in all body secretions and that its concentration in these secretions checks within normal limits with that of the blood urea nitrogen, both in normal individuals and in those with renal impairment. Little is said, however, as to the time element and pathologic conditions which may influence this diffusibility of urea nitrogen into body tissue.

Upon these facts as established by Marshall and Davis, and Myers and Fine, and other workers, Hench and Aldrich<sup>1</sup> started experiments on a secretion which was easily obtainable, namely, saliva, to determine whether or not its urea nitrogen content could be used as an index to the blood urea nitrogen, and therefore to renal impairment. Using the urease method they concluded that the average urea nitrogen content of the saliva is approximately 80 per cent that of the blood. Then, following the work of Friedlander, based on the mercury-combining power of saliva they developed a very time-saving and inexpensive technic for the determination of the salivary urea nitrogen which they showed could be used as an index to the blood urea nitrogen.

For our own satisfaction, we have checked this method in our laboratory, taking the cases as they presented themselves, regardless of diagnosis, to determine whether or not the blood urea nitrogen as determined by the salivary urea index and the actual blood urea nitrogen checked within sufficiently close limits to be used clinically, and whether or not the limits given by Hench and Aldrich as indicative of retention held for both normal and pathologic cases, for it has seemed to us that any condition which would disturb the functions of the rest of the body might also disturb the functions of

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the salivary gland or the diffusibility of urea nitrogen through this gland, and not necessarily in the same ratio

Hench and Aldrich say that "when a salivary urea index below 50 is obtained, there is, in nearly 90 per cent of the cases, no urea retention in the body. There is a 10 per cent possibility that mild retention exists, but not in any case does it exceed the blood urea value of 60 mg," and that "when a salivary urea index above 50 is obtained there is in 91 per cent of the cases definite urea retention, and only a 9 per cent possibility that there is no retention."

In our series, 75 estimations of normal and pathologic cases were made. The probable blood urea nitrogen as estimated from the salivary urea index and the actual blood urea nitrogen checked closely in the normal cases, as shown in Table I. The pathologic cases showed large and varied discrepancies between the probable blood urea nitrogen and the actual blood urea nitrogen. For convenience these cases have been divided into groups according to diagnosis and the findings tabulated in Table II which shows the number checking within normal limits, the number in which the actual blood urea

TABLE I

PROBABLE AND ACTUAL BLOOD UREA NITROGEN DETERMINATIONS IN NORMAL CASES

SALIVARY UREA INDEX	PROBABLE BLOOD UREA N MG PER 100 CC	ACTUAL BLOOD UREA N MG PER 100 CC
31	10.33	11.6
32	11.76	11.8
30	8.9	9.8
33	14.0	15.0
34	14.62	15.0
34	14.62	15.0
34	14.62	15.0
32	11.76	12.4
33	13.19	12.0

TABLE II

PERCENTAGE ERROR BETWEEN PROBABLE AND ACTUAL BLOOD UREA NITROGEN DETERMINATIONS IN NORMAL AND PATHOLOGIC CASES

DIAGNOSIS	NORMAL	DIABETES	HYPER TROPHY OF PROSTATE	NEPHRITIS	MYOCARDITIS	MISCELLANEOUS
Number of determinations	9	17	16	10	14	10
No. in which probable checked with actual blood urea N	9 (100%)	1 (10%)	--	--	3 (21%)	5 (50%)
No. in which probable was above actual blood urea N	1 (11%)	2 (20%)	4 (25%)	7 (70%)	6 (43%)	--
Per cent error	0 to 0.9%	29 to 183%	10 to 71%	23 to 180%	54 to 189%	--
No. in which probable was below actual blood urea N	8 (89%)	14 (70%)	12 (75%)	3 (30%)	4 (36%)	5 (50%)
Per cent error	0 to 1.4%	22 to 72%	10 to 200%	31 to 50%	14 to 48%	18 to 50%

nitrogen was below that estimated from the salivary urea index, the number in which the actual blood urea nitrogen was higher, and the percentage error in each group. In two of these cases the salivary urea was so high that it ran above the calibration of the flask so that the estimated blood urea nitrogen was 140 and 120 mg, the actual blood urea nitrogen in these cases was 75 and 58 mg.

TABLE III  
PROBABLE AND ACTUAL BLOOD UREA NITROGEN DETERMINATIONS IN CASES OF  
DEFINITE RETENTION

SALIVARY UREA INDEX	PROBABLE	ACTUAL	DIAGNOSIS	REMARKS
	BLOOD UREA N MG PER 100 CC	BLOOD UREA N MG PER 100 CC		
45	30.35	27.0	Prostatitis	
45	30.35	27.8	"	P S P 9½% in 2 hr
34	14.62	20.7	"	
38	24.34	36.0	"	
31	10.33	15.0	Pyonephrosis	P S P 33% in 1 hr
46	31.78	40.88	Diabetes	P S P 9½% in 2 hr

Table III shows the determinations made in the six cases of renal impairment with definite urea nitrogen retention. The percentage error between the probable and actual blood urea nitrogen determinations falls within allowed limits but in all of these six cases the salivary urea index was less than 50 which according to Hench and Aldrich would have indicated no urea nitrogen retention, allowing for a 10 per cent error. These six cases constitute 10 per cent of the 54 having a salivary urea index of less than 50, but we cannot agree that a method which would allow an error in six out of 54 cases is sufficiently accurate for clinical use.

#### CONCLUSIONS

1 In the normal individual, as shown by Hench and Aldrich and by ourselves, the probable blood urea nitrogen as estimated from the salivary urea index, and the actual blood urea nitrogen are in very close relationship.

2 In pathologic cases there is a wide variation between the probable blood urea nitrogen as indicated by the salivary urea index and the actual blood urea nitrogen. Apparently this variation follows no definite rule or pathologic condition, and this indicates, we believe, that with the pathology, other factors enter in such as the rate of diffusibility of the urea or a change in the tissue structure of the salivary gland. In some cases the saliva showed the presence of an albuminous substance which did not precipitate on boiling and apparently was at least partly responsible for the wide variations in the probable and actual blood urea nitrogen determinations since it was present in all but two cases showing wide variation.

3 The figures given by Hench and Aldrich as indicating the limit of normal urea nitrogen content of the blood are too high and may include many cases of actual urea nitrogen retention. The limits of tolerance are too great for accurate clinical work.

4 Finally, we conclude that the use of the salivary urea nitrogen determination as an index to the blood urea nitrogen content, as worked out by Hench and Aldrich can neither be used as a substitute for blood chemistry nor as an accurate indicator or selector of cases to be subjected to more complete tests

## REFERENCES

- <sup>1</sup>Hench, P S, and Aldrich, Martha A Salivary Index to Renal Function, Jour Am Med Assn, 1923, lxxxi, 1997 2003  
 Marshall, E K, and Davis E M Urea Its Distribution in and Elimination from the Body, Jour Biol Chem, 1914 xviii 53 80  
<sup>2</sup>Myers, V C, and Fine M S Comparative Distribution of Urea Creatinin, Uric Acid and Sugar in the Blood and Spinal Fluid Jour Biol Chem 1919, xxxvii 239 244

## GINGIVITIS, III\*

## LABORATORY METHODS FOR STUDY

BY ROBERT A. KELLY, M.D., WASHINGTON, D. C.

THE purpose of this paper is to report a method for the laboratory study of the gingivae and their sulci. It is one of a number of papers giving the details of different phases of this subject. The method, as outlined, is one used in the study of gingivitis, previously reported and by practical application may be adapted to the needs of the modern dentist as an assistance in diagnosis and treatment. The laboratory worker can be of tremendous help to the dentist by determining the character and amount of infection and infestation of the gingivae in a given case.

In the past this very important bacterial culture field, the gingival sulcus, has been neglected by the laboratory except by those men who have been especially interested in either obtaining material for a special study or in attempts to determine the etiology of "pyorrhea" (a term discarded for the more inclusive one of gingivitis). It will be necessary for clinical bacteriologists to familiarize themselves with this nest of infection and infestation and then to cooperate and study the problem with the assistance of a good modern dentist. It will be necessary to hold an open mind until the last of the problem has been conquered and nothing will be gained by fixed and finished opinions either as to what has been discarded in the past, what is now controversial, or what may be problematic for the future. I have said this to the subject several times during the past eight years but now, more than ever, regard it of prime importance. It is one that bids fair, the sooner it is recognized in its early stage, to counteract much future trouble for a large group of the people. It is necessary then for the laboratory worker first to become thoroughly familiar with the field, to educate some good dentist or group with whom he may work and then both cooperate for the benefit of the individual patient as

D C \*From the Laboratories of The Diagnostic Centre U S Veterans Bureau Washington

well as for the solution of the problem as a whole. This group study appeals to the internist, and with his complete cooperation the results obtained in the selected group of gingival focal infections are often striking.

In the study of infection and infestation of gingival exudates, I have made use of fresh and stained smears, various types of media, and pathologic sections where possible. When the patient is referred for the first examination, a complete report, made by the dentist, of the condition of the teeth and a complete dental x-ray is of great assistance. The report should call attention to mechanical dental conditions responsible for traumatic areas, the general dental survey and any teeth which are so called dead or devitalized. It is important to know just what, when, and how long treatment has been directed at the gingivae. The dental x-ray will immediately call attention to the degree of deep changes about the roots of teeth. While the history is of great aid, the character of the bacteriologic examination is entirely in the hands of the laboratory man, and he should make his own survey as he sees the field. He should not by any means merely carry out a routine order, examine for Vincent's spirillum or examine tooth No. 5 for the presence of streptococcus.

In the examination make a complete survey of the mouth noting the conditions briefly and describing in some detail the pathologic picture of the gingivae. This should include a brief description of the mouth as a whole, dentures, bridges, crowns, fillings, and dental hygiene. *It should not be and is not the field or duty of the clinical pathologist to note the mechanical features of the mouth, only in so far as they are related to the problem of infection and in so far as their correction is a means to an end in treatment.* Bridges, crowns, and dentures are secondary etiologic factors or negative phases in gingivitis and must be corrected before the infectious factors can be conquered. This is the problem of the surgical dentist but here cooperation with the laboratory is not only valuable but absolutely necessary. It is pretty hard on a patient who has recently had from eight hundred to fifteen hundred dollars worth of dental mechanical work, to be told that this must be sacrificed before a complete infectious problem can be controlled, but it must be done. This is an economic problem. I have recently seen two such cases, one of iritis and one of polyarticular arthritis where the correction of infectious factors compelled an entirely new dental mechanism. This is an educational problem on the side of the dentist and a big responsibility on the side of the pathologist. The responsibility for the latter is, first, the accurate recognition of existing conditions, and second, judgment in making recommendations.

It is a responsibility which should be divided between the dentist and the laboratory, since it is intimately concerned with dental mechanics and infectious factors. The sooner the laboratory takes an active interest in the dentist's problems the sooner the unnecessary sacrifice of teeth and life will be stopped, and the sooner the unnecessary trouble and expense of several dentures will be controlled by one proper outline from the beginning. Speaking of loss of life, hardly a month goes by that we do not hear of a case of cellu-

itis, septicemia, and death following a simple dental extraction. This sacrifice of life can be greatly reduced if not absolutely controlled when the infectious factors in the given case are studied and corrected beforehand. This is a statement based upon much experience with infection not corrected following dental operations, and without infection when adequately corrected following extensive dental surgery. The recognition of and preoperative treatment for gingival infections, reduces the postoperative reactions to a minimum and allows extensive surgery which would otherwise be impossible in the presence of infectious factors.

Following the survey of the mouth as a whole, the physical condition of the gums should be noted and if one follows the pathologic outline as described,<sup>1</sup> it is surprising how much can be seen. Especial attention is directed at the sulcal exudate with its character as a whole and with especial note about teeth under special consideration. This is an examination first of the field as a whole and second of individual teeth and their infectious factors. The x ray picture of a complete mouth before one at this examination is most valuable in directing attention to definite areas.

The bacteriologic character of gingival sulcal infection may be obtained by examination of smears, cultures, and tissue. The smears will give a great deal of information, and fresh smears will give more than stained smears. The dark field will permit character studies of individual organisms, but I do not believe it is necessary for routine use. The method of obtaining material and the examination of smears has been previously outlined. Study the general character of the infection present by a smear of a number of sulci and all sulci in the mild cases and follow this by examination of individual areas for special study. Three or four preparations may be necessary, the important point is not to miss anything and to make notation of all types of infection recognizable. The fresh smear is made in warm physiologic salt solution and the material is collected by a small loop 22 gauge chrome nickel wire in a holder. We have tried instruments, swabs, tooth picks, and capillary tubes but nothing is more convenient than the chrome nickel wire. Examine fresh smears immediately while they are warm (a warm stage is not necessary) under the high dry power, 4 mm objective. Make a notation of all types of bacteria and protozoa seen and indicate their prevalence by degrees of 1, 2, 3, 4. This is a very convenient record especially for purposes of treatment checkup. Fresh smears are made on flat glass slides with a coverslip. Concave slides are not necessary but may be used. I have recently been making concave slide cultures cemented with vaseline and paraffin and have found them convenient for incubator purposes.

Stained smears have not been found as instructive as the fresh smears, but are valuable in individual and special studies or for the predominance of bacterial types. The fresh smear may be used for the stained preparation by removing the cover glass, drying and fixing and staining by any method desired, Gram is one of preference. In the study of protozoa I make use of Schaudinn fixative and hematoxylin staining but this is not necessary for routine examination. All in all, the fresh smear may be relied upon routinely.

to give all the information necessary from the smear standpoint. It is rapid and there is no staining time delay. Where one sees a number of cases routinely, the factor of time is important. On the other hand when one begins the study of sulcal exudate, every method of examination will be found instructive.

In my paper last year<sup>2</sup> I said that the routine use of cultures was valuable but not necessary owing to the work entailed in examining a large number of cases. I would like to reverse this statement and now include the use of at least a blood agar plate and an anaerobic tube for the routine culture examination. This will entail a considerable burden where the daily examinations amount to any number of cases, but I believe the information obtained is worth the effort. Up until last year cultures were made in individual cases and in group studies but during the year I have routinely cultured every case on at least one blood agar plate and an anaerobic oxygen tension tube modified after Rosenow. This will be necessary until the bacteriologic flora of gingival sulci and its relationship to different types of gingivitis has been definitely established. That there is a relationship would seem to be borne out. There is no question of the advantage of cultural studies for preoperative cleanups. Animal or human, blood agar plates will grow all the coccical and bacillary flora and the same plate may be used for inoculation of several areas. We are still struggling with an adequate method for the cultivation of spirillumform organisms and have nothing to offer more than is in the literature at this time.

The study of gingival tissue cannot be carried on routinely owing to the obvious difficulty of obtaining material. It can be studied in all operative cases, and frozen sections with bacterial stains are very instructive. If the suggestion of Neuman, which I think is entirely too radical, that is, the stripping down of the gingivae of the entire mouth and allowing it to heal by granulation, was ever carried out, tissue would be available but I do not believe this will ever be the case. At this stage of our knowledge it is not necessary to study tissue except for special diagnoses such as granuloma, tumor, or tuberculosis.

Another appeal is made for the careful laboratory study on a large scale of gingival infections and their early recognition with the possible prevention of a trail of manifestations undoubtedly having their origin in the focal infection or portal of entry of a gingivitis. The magnificent work of Rosenow and many others has taken the subject of focal infection entirely out of the realm of faddism and possibility, and it remains for the future to detach it from the uncertainties now existing and firmly attach it to the certainties with which it is definitely and undoubtedly connected. The literature is filled with volumes and unfortunately many of the authors seem to have little or no first-hand information upon their subject. For this reason it is hoped to add in this series of papers some foundation to the clinical experience of the internist and modern dentist by a laboratory correlation.

## CONCLUSIONS

This paper deals with a routine method for the examination of gingival exudates with the idea of determining their bacteriologic characteristics

An appeal is made for a more widespread cooperation between the laboratory examination and the modern surgical dentist

The field belongs to both the clinical laboratory and the dentist, and there is the nicest chance for a cooperation which is worthy of any effort, since the results accruable to the welfare of the patient are much worth while

The examination should take into consideration the general character of the mouth, the teeth, the pathologic pictures of the gums, the gingival sulci, and the x ray picture with special reference to the periodontal areas

The gingival exudate is best examined by fresh smears and cultures Stained smears and dark field may be used for special studies

The gingival exudates should be examined by culture and the use of a blood agar plate and an anaerobic oxygen tension tube will determine the coccal and bacillary forms

The spirilliform organisms and protozoa are best determined by smear examination, and a satisfactory culture medium for these forms cannot be offered at this time

## REFERENCES

- Kelty, Robert A. Gingivitis, II The Clinical Appearance of the Gingivae in Different Forms and Stages *JOUR. LAB AND CLIN MED* to be published  
Kelty, Robert A. The Present State of our Knowledge of Gingivitis *JOUR. LAB AND CLIN MED* 1928 xiii No 5 451-468

## APPARATUS FOR SHAKING BLOOD COUNTING PIPETTES\*

By ROY F. FREEMSTER, M.D., D.P.H. NEW ORLEANS

IN OUR experimental work it has been necessary to make numerous blood counts on animals and consequently the shaking of the pipettes became quite burdensome. Because of this fact we were led to devise a simple apparatus to do this shaking, and it has proved very useful. Moreover our counts have become more accurate with the use of it. We obtain much better distribution of cells, for we tended to reduce the time of shaking when we had to undergo the discomfort of having the points of the pipettes digging into our fingers a number of times in succession.

The apparatus consists essentially of a light thin piece of poplar board to which the pipettes are securely fastened, and which is rapidly shaken by a small electric motor in such a way as to imitate the usual movements of the hand. Fig 1 will convey a sufficiently clear idea of the essential features that very little description is perhaps necessary.

Fig 1 A shows the method by which the pipettes are secured to the apparatus. A small wooden strip pierced by a number of holes just large enough



to give all the information necessary from the smear standpoint. It is rapid and there is no staining time delay. Where one sees a number of cases routinely, the factor of time is important. On the other hand when one begins the study of sulcal exudate, every method of examination will be found instructive.

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The spirilli form organisms and protozoa are best determined by smear examination, and a satisfactory culture medium for these forms cannot be offered at this time

## REFERENCES

- Keilty, Robert A. Gingivitis II. The Clinical Appearance of the Gingivae in Different Forms and Stages. *JOUR. LAB. AND CLIN. MED.* to be published  
 Keilty Robert A. The Present State of our Knowledge of Gingivitis. *JOUR. LAB. AND CLIN. MED.* 1928 xiii No 5 4 1468

## APPARATUS FOR SHAKING BLOOD COUNTING PIPETTES\*

BY ROY F. FELMSTED MD, DPH NEW ORLEANS

IN OUR experimental work it has been necessary to make numerous blood counts on animals and consequently the shaking of the pipettes became quite burdensome. Because of this fact we were led to devise a simple apparatus to do this shaking, and it has proved very useful. Moreover our counts have become more accurate with the use of it. We obtain much better distribution of cells, for we tended to reduce the time of shaking when we had to undergo the discomfort of having the points of the pipettes digging into our fingers a number of times in succession.

The apparatus consists essentially of a light thin piece of poplar board to which the pipettes are securely fastened, and which is rapidly shaken by a small electric motor in such a way as to imitate the usual movements of the hand. Fig 1 will convey a sufficiently clear idea of the essential features that very little description is perhaps necessary.

Fig 1 A shows the method by which the pipettes are secured to the apparatus. A small wooden strip, pierced by a number of holes just large enough

to admit the shaft of the pipette without allowing the bulb to pass through, is nailed to one side of the moving board. A row of metal clips, illustrated by Fig 1-B, hold the pipettes at the other side of the board. A rubber band, held at one end by a nail driven in the board, securely presses against the tip of the pipette and keeps the fluid from running out of the bulb during the shaking.

The board carrying the pipettes should be made as light as possible, as the lighter the board the less the vibration. The board is shaken by a bend in a shaft made of heavy wire to which a pulley is fixed. This pulley is

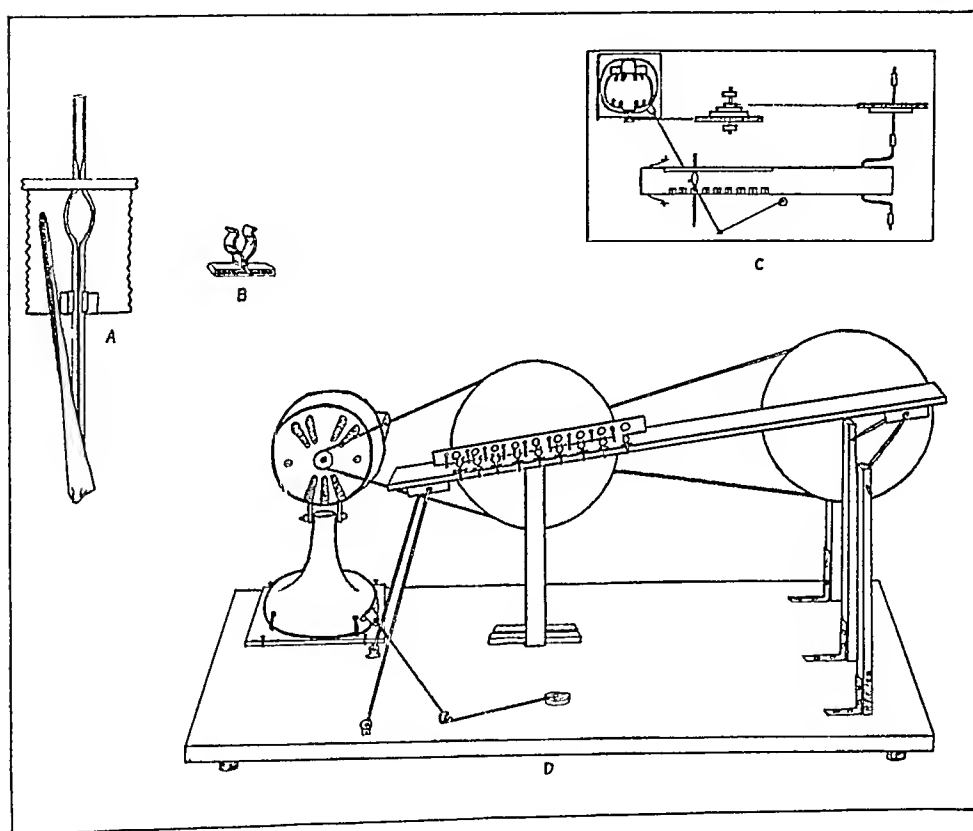


Fig 1

turned by a belt coming from a system of pulleys turned by another belt from the electric motor, the whole system being so adjusted that the moving board makes about 300 complete excursions per minute.

We made the pulleys we needed from white pine a little over a quarter of an inch in thickness. Circles of the desired size were cut out with a coping saw and a hole just the size to fit snugly on the shaft of the electric motor was drilled in the exact center of each. They were then mounted on the motor and allowed to turn rapidly, the groove in the circumference then being cut with a sharp instrument, just as on a turning lathe. However, metal pulleys can often be found about a laboratory on an old piece of apparatus.

Figs 1-C and D give two views of the arrangement of the parts of the

stain for the study of these cells Bergonzini (1891) studied the connective tissue of the mouse, rabbit, guinea pig, and frog, after staining in a combination of dyes consisting of a basic dye, methyl green and two acid dyes, namely, fuchsin and orange G Pappenheim's (1899) methyl green pyronin stain is well known as a very satisfactory preparation for staining mast cells Schridde (1905) stained with aniline water acid fuchsin and counterstained with an alcoholic solution of picric acid This method gives very clear figures when the combination of stains is properly used His figures are clear and show not only the granules of the cells sharply contrasted against the color of the cytoplasm but also the nuclear content is clearly differentiated Schaffer (1907) used three stains toluidine blue methylene blue, and thionin which are recognized as good basic stains for mast cells Perhaps one of the most popular stains among laboratory workers is the polychrome methylene blue of Unna (1910) Krause (1927) gives a very comprehensive review of staining methods used in the study of all types of plasma cells

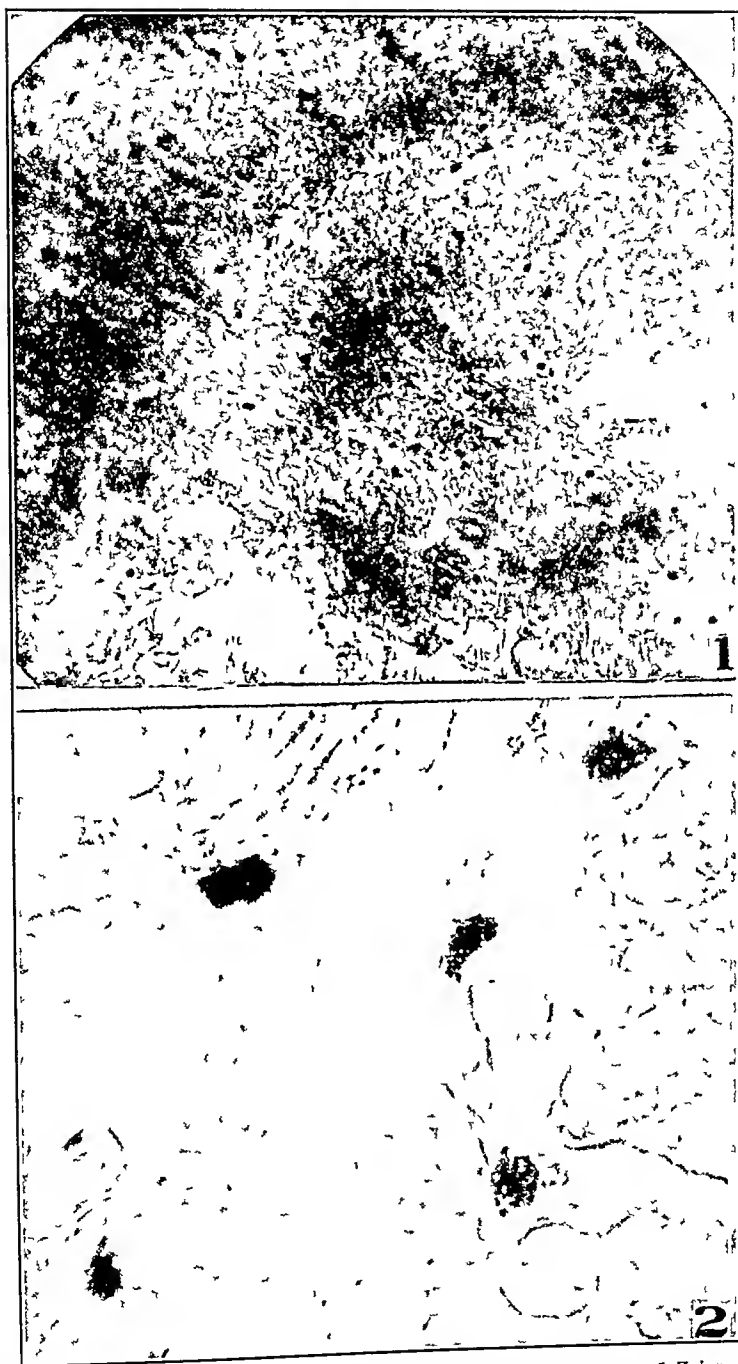
As mentioned above, I tried to stain paraffin sections of the parathyroid gland of various animals including man and I found that while the old stains are useful, they frequently fall short of their purpose Differentiation of the various cellular elements is frequently lacking Tissue elements other than the mast cells take up the dyes in the same degree and with the same rapidity so that recognition of the mast cells is difficult Mast cells with few granules (possibly transition stages), or very fine granules, are difficult to recognize

I have used the dyes employed by Schaffer (1907), especially thionin, in an aqueous solution to stain paraffin sections of the parathyroid glands of the steer, cow, rat, mouse, cat, rabbit, and man As a counter stain a saturated solution of orange G in oil of cloves was employed The mast cells with these stains, stand out prominently in blue or purple blue color while the background of connective tissue stains orange or gold color Transition stages are well defined The contrast between mast cells and nuclei of the stroma, especially in lymphoid tissue is well marked by differences in intensity of color The nuclei of the stroma tissue stains a very faint blue In deeply stained preparations the parenchymatous tissue of the parathyroid gland also stains sharply and is well differentiated The nuclei take on a brilliant light blue or purple color while the cytoplasm stains a faint orange

#### METHOD

A large number of fixations may be used The best results are obtained after fixing in one of the following solutions Alcoholic formal Bouin's picroformol acetic, Regaud's picroformol, Carnoy's, Zenker's, Flemming's weak and Flemming's strong, bichloride acetic, Orth's and Orth's plus acetic acid The sections may be cut from 5 to 10  $\mu$  The paraffin is removed by xylol in the usual way The slides are then washed in 95 per cent alcohol and immersed immediately in tap water for one to two seconds I omit the graded series of alcohols generally used after the xylol bath without injury to the tissue

Place the slides in a bath of 1 to 2 per cent solution of thionin for two to three minutes Wash off excess stain in tap water if light stain is desired For densely stained, markedly contrasted preparations prolong exposure to



Photographs were made with D and L. Bausch and Lomb camera and Zeiss lenses. Fig 1 was made with obj 16 mm oc K8 bellows 42 cm. Fig 2 obj 2 mm oc K6 bellows 43 cm.

Fig 1 shows a section of the parathyroid gland of a tuberculous cow. The number of mast cells in the stroma is very large in comparison with that observed in the human material and even larger than the number generally found in the rat or mouse. Fig 2 represents an enlarged portion of Fig 1 showing mast cells with distinct granular bodies in the cytoplasm.

thionin from ten to fifteen minutes. Omit washing the slide in water after taking it from the thionin bath. Remove the excess stain from slide about the sections with cloth. Dehydrate by dropping 3 to 5 drops of neutral absolute alcohol over the sections. The sections appear bluish purple to the naked eye. Immerse the slide in a concentrated solution of orange G in oil of cloves. After a minute or two depending on the thickness of the sections and density of the thionin stain, examine preparations under the microscope. Repeat the immersion in the solution of orange G until the thionin stain disappears from the cytoplasm of the connective tissue leaving it a golden orange color. The mast cells are unaffected and remain a deep purple or blue color. Long exposures up to twenty minutes in orange G do not remove the thionin stain of the granules of the mast cells. The parenchymatous tissue, however, becomes faintly orange except the nucleoli of the chief cells which retain a pale light blue coloration. The orange G goes into solution in oil of cloves slowly. This solution should be stirred frequently and allowed to stand for twenty four hours before using. The solution may be filtered although I have not found this necessary. The solution may be used for a long time before it becomes discolored by the thionin. After removing the slides from the orange G solution, wash thoroughly in xylol and mount in Canada balsam. The thionin may be substituted by methylene blue, toluidine, gentian, and dahlia. More uniform results have been obtained with thionin.

Examination of a section of the parathyroid gland of the cow stained by this method shows that the cytoplasm takes on a faint orange color and only the nuclei of the chief cells retain a light blue stain of the thionin. The nucleoli stain heavier. The mast cells are clear and readily recognizable with low magnification. Under high magnification the nuclei of the mast cells show deeply stained peripherally distributed chromatic bodies. The granular bodies of the cytoplasm retain a deep purplish color. The granules in a single cell are of various sizes and are of a homogeneous consistency. Occasionally the larger granules have a clear central area with a thin peripheral layer of stainable material. In the parathyroid of the cow I have observed the presence of a small number of mast cells which have very fine granules of uniform size.

#### SUMMARY OF METHOD

- 1 Fix material in any of the usual killing agents
- 2 Imbed in paraffin
- 3 Section material 5 to 10  $\mu$
- 4 Remove paraffin in xylol
- 5 Remove xylol in 95 per cent alcohol
- 6 Rinse in water
- 7 Stain in 1 to 2 per cent thionin in water
- 8 Remove excess stain in water, remove water about sections, dehydrate
- 9 Stain in solution of orange G in oil of cloves
- 10 Observe preparation under microscope until desired results are obtained
- 11 Wash thoroughly in xylol
- 12 Mount in Canada balsam

## REFERENCES

- <sup>1</sup>Bergonzini, C    Über das Vorkommen von granulierten basophilen und acidophilen Zellen im Bindegewebe und über die Art sie sichtbar zu machen, *Anat Anz*, 1891, vi, 595 600
- <sup>2</sup>Ehrlich, P    Beiträge zur Kenntnis der Anilinfärbungen und ihrer Verwendung in der mikroskopischen Technik, *Arch f mikr Anat*, pl 21, 1877, xii, 263 277
- <sup>3</sup>Krause, R    Enzyklopadie der Mikroskopischen Technik, 1926, ii, 1355, 1927, iii, 2066, 2188
- <sup>4</sup>Pappenheim, A    Vergleichende Untersuchungen über die elementäre Zusammensetzung des rothen Knochenmarkes einiger Säugethiere, *Virehow's Arch f path Anat*, taf 2, 3, 1899, clvii, 19 76
- <sup>5</sup>Schaffer, J    Präparate von lockerem Subkutinalgewebe von der weissen ratte, *Centralbl f Physiol*, 1907, xxi, 261 262
- <sup>6</sup>Schridde, H    Die Kornelungen der Plasmazellen, *Anat Hefte*, taf 26, 1905, xxi, 691 768
- <sup>7</sup>Unna, P G    Enzycl Mik Techn, 1910, vi, 72

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## METHOD OF ELIMINATION OF THE ANTICOMPLEMENTARY RESULT IN MANY WASSERMANN TESTS\*

BY HAMILTON R FISHBACK, M D, Sc D, CHICAGO

THE occurrence of an anticomplementary result in a Wassermann test is as unsatisfactory to the serologist as it is vague to the clinician, who is often misled into considering such a result as indicating a positive Wassermann. Carefully controlled and improved technique in performing the test and care of the blood sample itself have eliminated a large percentage of such blurred results. However, the drawing and preservation of blood samples from sources outside the laboratory or hospital often cannot be controlled. In addition to the above mentioned ordinary causes of difficulty are those which may be inherent in the serum itself.

Numerous observations have been made upon anticomplementary substances in Wassermann sera, either as a result of direct study, or incidentally in the course of general Wassermann work. A résumé of the literature is not considered essential in this presentation.

The following method of testing anticomplementary sera has been used, under my direction, at the Wesley Memorial Hospital of Chicago for the past three years. The Wassermann technique in general is carried out according to the Cleveland method†. In those tests in which absorption of complement is found in the control, the serum is subjected to a series of dilution tests. In a series of approximately ten tubes, the amount of serum used for each test varies from 0.1 cc to 0.005 cc, with a constant amount of complement and antigen, and a constant hemolytic system. A control is included for each dilution. The amounts of serum used, and the consequent number of tubes, may be varied to the right or left in the type set-up as given, according to the strength of the anticomplementary reaction in the original test. The antigen

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†C L Cummer Manual of Clinical Laboratory Methods, ed 2 p 164 1926 Lea and Febiger.

TYPE SET-UP OF TEST

SERUM (c.c.)	1	2	3	4	5	6	7	8	9	10
	0.1	0.08	0.06	0.05	0.04	0.03	0.02	0.01	0.007	0.005

Remainder of Wassermann test materials constant in amount

RESULTS

Positive Test	4	4	4	4	4	4	3	3	2	0
Control	4	4	3	2	1	0	0	0	0	0
Negative Test	4	4	4	3	2	1	1	0	0	0
Control	4	4	3	2	1	0	0	0	0	0

dose used is 0.1 c.c. of a 1:50 dilution, per tube, with an anticomplementary unit of 0.1 c.c. of a 1:5 dilution, when used with inactivated negative serum.

Two type results are given. In the positive, the fixation of complement persists in much smaller amounts of serum in the test than in the control. In the negative, there is approximate agreement of lessened complement binding in both test and control with decreasing amounts of serum. The individual results of test and control vary, of course, with every serum, but the curves show the general relationship given in the type results.

In the course of three years in general laboratory work anticomplementary elimination tests have been performed upon various kinds of sera. Sera with rich and varied bacterial flora, some chemically contaminated in ordinary unclean bottles, others showing deterioration after exposure to air, or long standing, or mechanical trauma, these have all been subjected to the given procedure. In addition many have been tested which in the fresh state were anticomplementary from unknown reason.

It is evident that, theoretically, if a weak syphilitic reagin be associated with a strong, nonspecific complement binding substance, the latter may persist while the former is titrated out. Two plus sera, by the routine test, were inoculated with staphylococcus and incubated one to three days. Strongly anticomplementary sera resulted, which could be titrated down, leaving a definitely positive test and clear control, with a few exceptions. No solution is offered here for these exceptions, or for sera of still lower Wassermann value in which indefinite results were obtained.

It is well known that anticomplementary substances may exert slightly more binding power in the presence of antigen. The strength of this effect is not sufficient, however, to alter titration values significantly, and it does not obscure the reading of results. Both thermolabile and thermostable anticomplementary substances are eliminated by the method.

Conclusion. A method is outlined for the elimination by titration of anticomplementary substances in many Wassermann reactions.

This test was run in conjunction with the Cleveland Wassermann method.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## CLINICAL AND EXPERIMENTAL

**LOBAR PNEUMONIA** A Clinical and Bacteriologic Study of 2000 Typed Cases, Cecil, R. L., Baldwin, H. S., and Larsen, N. P. Arch. Int. Med., 1927, xl, No. 3, p. 253

A summary of the more important facts derived from this investigation may be stated as follows:

A bacteriologic classification of 2,000 typed cases of lobar pneumonia showed the following distribution of pathogenic organisms: pneumococcus, 95.65 per cent, hemolytic streptococcus, 3.8 per cent, Friedländer's bacillus, 0.4 per cent, influenza bacillus, 0.05 per cent, and Staphylococcus aureus, 0.1 per cent.

The incidence of the various types of pneumococcus was as follows: pneumococcus type I, 33.6 per cent, pneumococcus type II, 19.2 per cent, pneumococcus type III, 13.4 per cent, pneumococcus type IV, 33.1 per cent. There was considerable variation from year to year in the incidence of the various types.

A study of the relation of types to age shows that young people are particularly prone to pneumococcus type I infections, and elderly people to pneumococcus type III. Type II and type IV show no special predilection for any age group.

A study of the incidence of type in relation to sex shows that type I and type II are commoner in men than in women. Type III and type IV are commoner in women than in men. Type III is almost twice as common in women as in men. Type I pneumonia is three times as common in men under 50 as it is in women over 50. On the other hand, type III pneumonia is more than three times as common in women over 50 as it is in men under 50.

The percentage of mixed infections among the cases of pneumococcal pneumonia is lowest in type I cases (18.4 per cent) and highest in the type IV group (31.2 per cent). The incidence of mixed infections varies from year to year, being highest during years when influenza is prevalent. By far the commonest mixed infection was that of the pneumococcus in association with the influenza bacillus. The pneumococcus and hemolytic streptococcus is the next most frequent combination.

The incidence of preexisting systemic disease is highest in the pneumococcus type III group, 63.3 per cent of all type III infections occurring in patients with systemic disease.

The type of pneumococcus appears to bear no relation to the lobe involved or to the extent of the infection. Three and four lobe infections are as relatively frequent in the milder types (I and IV) as in the severe types (II and III).

Twenty-four per cent of the cases of pneumococcal pneumonia showed pneumococci in the blood. The incidence of bacteremia is higher in the severe types (II and III) than in the mild types (I and IV). Type II is essentially the pneumococcus of bacteremia, yielding positive blood cultures in 39.7 per cent of the cases.

No striking differences in the clinical course could be demonstrated as characteristic of the various types of pneumococci. The average duration of the disease for the various types ranged from 8.6 to 9.1 days, type III running the longest course. Type III infections also ran the longest courses in monkeys.

A study of the mode of termination in the cases of pneumococcal pneumonia in which the patients recovered showed that 49.6 per cent terminated by crisis. Crisis was most frequent in type I infections, 56.6 per cent, type II, 50 per cent, type III, 39.3 per cent, type IV, 45.5 per cent.

The death rates for the various types (without specific treatment) were as follows: type I, 20.7 per cent; type II, 4.2 per cent; type III, 41.6 per cent; type IV, 29.2 per cent. Types I and IV are the milder forms; types II and III are the severe forms of pneumococcal pneumonia.

A study of the death rate in relation to age shows a steadily increasing death rate for each decade. In patients under 20 the death rate for all types was 11.1 per cent; in those over 60, the death rate was 52.7 per cent. Age is probably the most important single factor in determining the death rate in pneumonia. *Incubococcus* type I pneumonia has a low death rate, presumably because it is the pneumonia of young people. *Pneumococcus* type III pneumonia has a high death rate, chiefly because of its prevalence in elderly people. In patients under 30, type III pneumonia is not a severe infection.

The relation of bacteremia to death rate was striking in all four types. Among 240 patients whose blood remained sterile the death rate was only 18.7 per cent; among eighty-nine patients whose blood cultures were positive the death rate was 83.1 per cent.

The association of some systemic disease with pneumonia increases the death rate. Among patients free from systemic disease the death rate was 34 per cent; among those with systemic disease, the death rate was 46.4 per cent.

In all four types of pneumonia the death rate was definitely related to the number of lobes involved. For single lobe infections it was 20.9 per cent; two lobes, 36.3 per cent; three lobes, 40.8 per cent; and four lobes, 65.5 per cent.

Some form of pneumococcal complication occurred in 17.1 per cent of type I cases. The incidence of pneumococcal complications was almost twice as high for type I infections as for the other three types.

Empyema was the commonest complication, occurring in 5.1 per cent of all cases. The incidence of empyema was considerably higher in type I infections than in any of the other types. Empyema is a much more serious complication when it develops during the active stage of pneumonia than when it occurs after the crisis.

### VAGINITIS Clinical Criteria for Differential Diagnosis of Nongonorrheal Infantile Vaginitis Tomasi L. Pubeln Soc. Prat. 1921, xxxiv, 415

#### Acute

##### Gonorrheal

Acute stage rather marked and short. Creamy whitish yellow or yellow greenish abundant discharge.

Swelling and reddening of external genitalia and surroundings.

Involvement of the urethra.

Pus with characteristic intercellular gonococci, gram negative, few epithelial cells and leucocytes.

Vagina upper vagina involved, erosions, hemorrhagic spots.

##### Nongonorrheal

Relative acuteness short duration. Scarce liquid leucorrheal or mucous, sometimes serous discharge with solid and rice water features.

Perigenitals not inflamed, except in ulcerativeness.

Urethra not involved.

Fibrin and epithelial cells, more or less pus cells, vacuolized abundant mixed flora cocci, bacilli.

Lower vagina involved, no erosions, vivid reddening, hemorrhage, diffuse, no gonococci.

#### Subacute and Chronic

##### Gonorrheal

Sudden onset, more or less acute recurrences.

Pus with intra and extracellular diplococci, gram negative and positive diplococci and bacilli.

Urethritis.

Repetition of acute symptoms

Repeat examinations in acute stage

Limited to upper vagina, discharge, hemorrhagic spots, papillomatous appearance of mucosa

Frequent involvement of cervical canal, pus, mucus, hemorrhage

Endocervical examination

Provocation with vaccine brings on recurrence

Cutereaction and intradermal reaction produce inconsistent results

Complement deviation in about 50 per cent positive

#### Nongonorrheal

Insidious painless onset, not acute, varying degree of discharge

Semblance to old gonorrhea, gram test decides

No urethritis

Varying acuteness, absence of acute recurrences

Often scrofulous lymphatic appearance, velvety papules

Cervical Canal Often granular aspect, but no discharge

Provocation with vaccine not effective, local chemical causes slight reactivation

Cutereaction produces positive Pirquet, or symptoms not in keeping with vaginal condition

Complement deviation (gonococcal antigen) rare as specific results

**PULMONARY TUBERCULOSIS** The Hemotonic Index and the Resistance of the Red Corpuscles in Pulmonary Tuberculosis, Secco, C II Policlinico, July, 1926, xxxiii, 1041

The hemotonic index is expressed by the equation  $\frac{T}{G + Hb}$  in which Hb = hemoglobin,

G = red cell count, and T = the maximum arterial pressure in mm

Normally the index is between 3 and 4

Secco finds the results of both this index and the red cell fragility test of no prognostic value in tuberculosis

**BLOOD POTASSIUM** A Colorimetric Method for the Determination of Potassium With 0.2 cc of Blood, Yoshimatsu, S Tohoku Jour Exper Med, 1926, viii, 174

#### Preparation of Reagents

##### 1 Sodium cobalt nitrite reagent

*Solution A*—25 grams of cobalt nitrate crystals are dissolved in 50 cc of water, and to this solution are added 125 cc of glacial acetic acid

*Solution B*—120 grams of sodium nitrite (potassium free) are dissolved in 180 cc of distilled water To the whole of solution A are added 210 cc of solution B Air is drawn through the solution until all the nitric oxide gas has passed off The reagent thus prepared is kept in an ice chest and filtered each time before using It will keep at least one month

##### 2 Potassium cobalt nitrite

Adding slowly 300 cc of sodium cobalt nitrite solution (1) to 50 cc of 1 per cent pure potassium chloride, the potassium cobalt nitrite settles rapidly

The precipitate is transferred to a Buchner funnel, washed three or four times with cold distilled water until the last washing fails to take color when a drop of ammonium sulphide is added And then air dried on the Buchner funnel

##### 3 Diluted nitric acid

To 117 cc of concentrated nitric acid (spec w 1.317) is added 283 cc of distilled water

##### 4 Standard solution

0.2905 grams of potassium cobalt nitrite (2) is dissolved in 250 cc of diluted nitric acid (3) by warming, then made up to 500 cc exactly with distilled water

##### 5 1 per cent alcoholic solution of dimethylglyoxim

- 6 1 per cent sodium sulphide solution
- 7 Glacial acetic acid
- 8 Sodium acetate (powdered)

### Procedure

0.2 cc of blood is dried in a platinum dish or crucible over the steam bath or in an incubator at 110° C. Then the dish or crucible is placed in a large porcelain dish (6 or 7 cm in diameter) with pieces of porcelain on the bottom. The porcelain dish and its contents are heated over a low flame until no more fumes come off. Then the porcelain dish with the crucible remaining inside is covered with a quartz or porcelain plate and now heated over a large burner until the blood is completely ashed. The platinum dish or crucible is removed and allowed to cool. The ash is dissolved in 0.5 cc of water with one or two drops of glacial acetic acid (7). 0.5 cc of sodium cobalt nitrite reagent (1) is added drop by drop with constant shaking and then allowed to stand for at least 15 minutes. The precipitate is transferred into the centrifuge tube with the aid of a little water, and centrifuged at a speed of 2000 revolutions per minute for 5 minutes.

The supernatant fluid is decanted and 5 cc of distilled water poured into the tube which is centrifuged again. This procedure is repeated three times. After the last washing is decanted, 2 cc of distilled nitric acid (3) is added and warmed until the precipitate is dissolved. (Then follows the colorimetric technique below described.)

### Direct Determination of Potassium in Serum

According to the technique of Kramer-Tisdall the potassium is precipitated in undiluted serum by adding an excess of sodium cobalt nitrite reagent.

*Details of the Procedure*—1 cc of serum is put into a graduated centrifuge tube of 10 or 15 cc. 2 cc of sodium cobalt nitrite reagent (1) are then added drop by drop with constant stirring. At the end of 45 minutes 2 cc of water are added and the contents are again mixed and then centrifuged 15 minutes at a speed of 2000 or 2500 revolutions per minute. The supernatant fluid is decanted by means of centrifugation as above described, and the precipitate washed three times with water. The precipitate is then dissolved in 2 cc of diluted nitric acid.

*Colorimetric Technique*—15 grams of sodium acetate are put into a 50 cc volumetric flask and then the potassium cobalt nitrite from blood or serum dissolved as above mentioned is poured together with the remainings of the centrifuge tube. 40 cc of standard solution (4) (20 cc for serum) are pipetted into another 50 cc volumetric flask already containing 15 grams of sodium acetate. To both flasks about 20 cc of water, 5 cc of 1 per cent dimethylglyoxime solution (5) and 2 cc of 1 per cent sodium sulphide are added. After 3 minutes both flasks are put in an incubator (110° C) and kept there for 15 minutes and are then cooled, or they may be dipped into boiling water up to the depth of the neck and kept there for 15 minutes and then cooled in running water. Then water is added up to the mark for colorimetric determination. The colors of the contents of both flasks are compared in a Dubosq colorimeter, the standard being set at 20 mm.

Calculation is as follows:

$$\frac{\text{Reading of standard} \times 0.4 \times 500}{\text{Reading of unknown} \times 0.2 \times 100} = \text{mg of K in 100 cc blood.}$$

$$\text{Reading of unknown} \times 0.2 \times 100 = \text{mg of K in 100 cc serum}$$

## LABORATORY TECHNIC

NEUROGLIA FIBERS A New Method of Staining Salts T. Lo Orienta Bnl Neuro Biol, 1927, 1, 89

*Method A*—Fresh material is fixed in a solution of alum of fluorine (or chrom) 2.5 parts, acetic acid 5 parts, acetic oxide salt of copper 5 parts and distilled water 98 parts, for one or more weeks. Formolin 5 parts is sometimes added to the solution.

Frozen sections 5 to 10 microns thick are put into a solution of (a) 5 per cent sulphite

of soda 5 cc, distilled water 98 cc, and 40 per cent caustic soda 5 drops, or (b) 5 per cent anhydrous sulphate of soda 5 cc, 5 per cent bisulphate of soda 3 cc and distilled water 98 cc, for 10 minutes. They are then treated with a 0.2 to 0.5 per cent silver nitrate solution in darkness for 1 to 3 days.

The sections are then rinsed and placed in a reducing bath prepared as follows. Two drops of a 40 per cent caustic soda solution are placed in a graduate glass of more than 20 cc volume and covered with 3 cc of a one per cent silver nitrate solution. A concentrated solution of caustic ammonia is added drop by drop with constant shaking until the precipitate is dissolved. Add another drop of ammonia after complete dissolution. The vessel is then filled up to the 20 cc mark. The sections remain in the bath about 15 minutes. As soon as they begin to turn brown, they are transmitted into distilled water and into a solution of equal parts of 20 per cent neutral formalin and distilled water. Within 30 to 45 seconds the sections again become dark brown.

Gilding takes place slowly in a 0.1 per cent gold chloride solution into which a minimum amount of acetic acid may be added. To differentiate the neurofibrils the time of the gold bath is shortened, for the neurofibrils stain bright brown in contrast to the dark green of the glia fibers.

Dehydration and mounting are done as usual.

Counterstain may be done with lithium carmine, van Gieson fluid, scarlet red or Sudan III.

**Method B**—Fresh material is put into a solution of 4 per cent quinine sulphate 45 parts, 4 per cent ammonium bromide 45 parts and formalin 8 parts, for 2 days. Frozen sections are transmitted into the "b" fluid of Method A for 12 hours, rinsed and mordanted in Weigert's routine fluid for from 2 to 4 days. Further treatments follow those of Method A, except that the gilding is omitted since the glia fibers appear dark against the yellowish brown of the other tissues.

### AGGLUTINATION A Rapid Method for the Macroscopic Agglutination Test, Noble A Jour. Bact., 1927, xiv, No. 5, p. 287

For the standard method. One half cubic centimeter suspension plus 0.5 cc diluted serum, in agglutination tubes (90 x 8 mm, tapered at the end). Mix well, incubate at 37° C for eighteen to twenty-four hours.

For the rapid method. One tenth cubic centimeter suspension (containing five times as many organisms per cubic centimeter) plus 0.1 cc diluted serum (five times more concentrated than for the standard method) in small test tubes (75 by 13 mm). Shake two minutes and add 0.8 cc saline to make the total volume 1 cc.

Thus we have the same amount of serum and the same number of organisms in each test, for example

In the standard method 0.5 cc of serum diluted 1:50 contains 0.01 cc of undiluted serum  
In the rapid method 0.1 cc of serum diluted 1:10 contains 0.01 cc of undiluted serum

Because the majority are in the habit of thinking of agglutination titers in terms of dilutions this may be called a dilution of 1:100, meaning that there is actually present 0.01 cc of undiluted serum, regardless of the total volume.

Also, in the standard method 0.5 cc of suspension containing 2 x organisms per cubic centimeter = x organisms

In the rapid method 0.1 cc of suspension containing 10 x organisms per cubic centimeter = x organisms

The suspension containing 2 x organisms must be of such density as will give maximum agglutination by the standard method with a given organism.

**CARBON MONOXIDE** Chemical Tests for This Gas in the Blood Jones W C, and Pinkston, J O New Orleans Med and Surg Jour, 1927, lxxv, No 2, p 99

*Phenol Alone*—1 Undiluted To undiluted blood in test tubes add 95 per cent phenol in the proportion of 2 to 6 drops to each cubic centimeter of blood and mix thoroughly Carbon monoxide blood turns a brilliant pink and normal blood a very markedly contrasting dark chocolate The immediate results are a little better with the larger proportions of phenol The colors usually become a little more prominent on standing a few hours to a few days and persist strikingly for many days

*Diluted Phenol Test*—Equal parts of 5 per cent aqueous solution of phenol and undiluted blood yield a very satisfactory result The CO tube turns a marked red and the normal one a blackish brown The reaction is immediate and persists many days to several weeks The CO blood coagulates incompletely while the normal blood becomes entirely solid In the tests in which the blood is well diluted either directly or by the addition of diluted reagents, it is well to make the observations both by transmitted light and by light reflected from a white surface, if or as long as the contents remain fluid

*Alum Alone*—To a selected quantity of undiluted blood add an equal amount or one half as much of a saturated aqueous solution of aluminum potassium sulphate (alum) and mix thoroughly The blood coagulates, solidifying the contents of both tubes in a few minutes When the test is first made the red color of the CO tube is but little different from that of the normal but on standing a few minutes to an hour the positive tube turns a cherry red color and the normal one, a dirty blackish brown The contrast is very marked Do not expect this test to show positive results at once It requires a few minutes to an hour for the red of the normal tube to fade sufficiently so that the red in the other tube will stand out by contrast If however a quantity of water is added equal to the amount of blood used, the contrast between the two tubes appears almost at once, but it is not quite as marked as that which is seen ultimately in the tubes with undiluted blood It is interesting to note that the tubes to which this amount of water is added solidify as promptly as those without any water (except that which is included in the alum solution)

**LYMPH NODES** Method to Obtain Material by Puncture of Lymph Nodes for Study With Supravital and Fixed Stains Arch Int Med 1927 xl No 4 p 552

The procedure which has been developed and utilized in studies on lymph nodes is as follows The skin overlying the structure to be punctured is cleaned surgically by scrubbing with soap, followed by the application of ether and iodine or by mercuric chloride or picric acid solutions About 0.5 cc of procaine hydrochloride in solution is infiltrated through a small hypodermic needle into the skin A 17 or 18 gage sterile needle about 3.5 cm in length is then inserted through the anesthetized area until the point pierces the capsule of the lymph node or diseased tissue The exact position of the needle point is easily determined by the resistance it encounters and by the movability of the mass After the needle has been passed just within the tissue from which material is desired the needle is then steadied and a sterile coarse dental broach is passed through the lumen These broaches are constructed so that they enter the tissue like a needle but come out like a fish hook carrying on the barbs numerous elements of living tissue Smears of the material obtained are then quickly made on glass coverslips One puncture of a tumor or node yields sufficient material for several smears The more cellular the tissue the more material is obtained from which cultures as well as smears may be made After sufficient material has been obtained by inserting one or more broaches, the needle is withdrawn and a small dry sterile dressing applied The procedure seems to be no more painful than puncturing a vein in the arm for the withdrawal of blood, and there has been no difficulty in making repeated punctures of lymph nodes on the same patients

*Technic of Vital Staining*—A thin film of dye in alcoholic solution is smeared across the surface of a previously cleaned, polished and warmed glass slide This is done by dipping the end of another slide, with which the smear is made on the former one, into a fresh

mixture (10 drops) of equal parts of 0.5 per cent alcoholic solution of neutral red and a 0.1 per cent alcoholic solution of Janus green. Solutions of the dyes are kept on hand, made up in 95 per cent alcohol. The dyes are not mixed until they are ready for use, because a mixture is not a stable preparation. Solutions of varying concentrations should be employed for making the dye films until one has gained proficiency in estimating the optimum concentration desired. Emphasis should be placed on staining the cells as lightly as is consistent to obtain good differentiation of the structures they contain. If the dyes are employed in concentrated solutions, the nuclei will be stained and the cells quickly killed. The glassware must be scrupulously clean because cells are delicate structures, and any acid, alkali or the like will injure their membranes and markedly alter their physiologic behavior.

*Method Modified for Tissue Cells*—In the study of cells from blood, a free flowing drop of blood is procured on the under surface of a glass coverslip, and this is allowed to fall gently on the slide prepared with the dye. In the case of blood, the plasma takes up some of the dye and acts as a medium in which the cells maintain their activity in suspension. However, material obtained from nodes, especially if fibrosed, often has insufficient fluid to permit cells to be properly suspended. The addition of physiologic sodium chloride alters the staining qualities and behavior of the cells, and is not ideal for this purpose. As a fluid in which to suspend the cells one can use the patient's serum or that from a person belonging to iso agglutination group I (Moss classification) whose serum does not agglutinate red blood corpuscles. It is essential to have a fluid medium in which to suspend the cells immediately after their removal from the body in order to prevent desiccation and to promote a uniform staining reaction.

#### BLOOD CHEMISTRY Blood Chloride Methods, Short, J. J., and Gellis, A. D. Jour Biol Chem, 1927, LXXIII, No. 1, p. 219

The objections to several blood chloride methods are pointed out, with special reference to the method of Whitehorn. The end point in the Whitehorn method is unsatisfactory, as it yields results which usually are too low. The authors' technique, an adaptation of the McLean Van Slyke iodometric principle to the Fohn-Wu tungstic acid filtrate, is described. This is believed to be preferable to the Volhard titration. Tabulated results of a comparison of the methods of Austin and Van Slyke, of Whitehorn, and the proposed method are given. The proposed method possesses the advantages of being short, simple, and accurate.

The method follows:

Sodium Tungstate—A 10 per cent solution

Sulphuric Acid—a 2/3 N solution

Silver Nitrate—M/29.25 Dissolve 5.812 mg. of  $\text{AgNO}_3$  in 600 cc. of water in a 1 liter flask, add 250 cc. of  $\text{HNO}_3$  (sp. gr. 1.42). Dilute to mark with water.

Potassium Iodide—M/73.1 Dissolve 2.4 mg. of KI in 1 liter of water. Standardize by titrating against 5 cc. of the  $\text{AgNO}_3$  solution to which have been added 5 cc. of water and 6 cc. of starch indicator. Adjust so that the amount required will be 12.65 cc. (12.5 cc. to precipitate the  $\text{AgNO}_3$  and 0.15 cc. to develop a definite end point).

Starch Indicator—(A) Dissolve 2.5 gm. of soluble starch in 100 cc. of boiling water. Cool and dilute to 150 cc. (This solution should be prepared fresh each week.) (B) Dissolve 466 gm. of crystalline sodium citrate and 20 gm. of sodium nitrate in about 800 cc. of water. Dilute to 1350 cc. Solutions A and B are mixed in necessary quantities before determining chlorides, in the proportion of 1 to 9 respectively.

#### Procedure

To 1 volume of oxalated blood or plasma (at least 3 cc.) add 7 volumes of water and mix. Then add 1 volume of the 10 per cent sodium tungstate solution and 1 volume of the 2/3 N  $\text{H}_2\text{SO}_4$ . Shake. After the mixture has changed to a chocolate brown color, filter. To 20 cc. of the filtrate in a 50 cc. centrifuge tube, add 20 cc. of water, 10 cc. of the  $\text{AgNO}_3$  solution, mix by stirring and centrifuge for about 5 minutes in a high powered centrifuge.

Decant through a small filter paper 20 cc of this filtrate are then titrated with the standardized potassium iodide solution, using 6 cc of the starch indicator. The end point is a permanent deep blue color. Calculation (10.15 cc of KI solution used)  $\times 100 =$  mg of NaCl in 100 cc of blood or plasma

**TUBERCULOSIS** A New Stain for Tubercle Bacilli Elvers C F Jour Urol 1927, xvii, 573

The film on the slide is made in the usual way fixed by heat and placed for five minutes in a Coplin jar containing the following stains

Absolute alcohol	20 cc
Melted Phenol Crystals	20 cc
Basic Fuchsin	12 grams
Xylol (C P)	90 cc

Decolorize by gently rinsing in 0.25 per cent acid alcohol. Rinse for a few seconds in distilled water, air, dry and counter stain with Loeffler's methylene blue.

The results are very satisfactory. The cells and mucosa shreds are not curled or destroyed, and the field is free of all artefacts. The acid fast bacilli are stained red by the fuchsin, other organisms and cells blue. If this stain be kept at incubator or low oven temperature it will be more penetrating and give greater contrast after several weeks than when freshly made.

**SPINAL FLUID** Application of the Combining Power of Proteins With Rose Bengal as a Quantitative Test on the Spinal Fluid Rosenthal S M and Ackman F D Arch Int Med 1926, xxxviii, 527

The factors of error can be reduced to a negligible quantity by employing protein dilutions greater than 0.1 per cent. The normal protein content of the spinal fluid is only 0.025 per cent and in disease it rarely increases beyond 1 per cent. In the technique employed the lowest dilution of cerebrospinal fluid is 1:25 which in terms of protein content gives a dilution of 1:100,000 (0.001 per cent) in normal fluids. The following technique has been adopted. 1 cc of spinal fluid is diluted to 25 cc with distilled water. This is placed in four test tubes as follows

	Tube 1	Tube 2	Tube 3	Tube 4
Quantity of 1:25 cerebrospinal fluid - -	10 cc	5 cc	2.5 cc	1 cc
Water - - - - -	0	5 cc	7.5 cc	9 cc

To each tube 1 cc of 0.02 per cent rose bengal in aqueous solution should be added, and mixed by inverting the test tubes. To each tube 1 cc of tenth normal hydrochloric acid is now added and the solutions again mixed. Comparison of the color which remains should be made in a colorimeter with a standard prepared by adding 1 cc of 0.002 per cent rose bengal to 11 cc of water.

The  $P_H$  of the acidified solutions will be 2.01 to 2.2, at which acidity all of the free rose bengal will be decolorized, so that the color of the solution represents the bound dye. If a colorimeter is not available a series of standards may be prepared as follows. 10 cc of 0.02 per cent rose bengal is added to 110 cc of water into which 1 drop of 10 per cent sodium hydroxide has been placed. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 cc of this solution are placed in ten test tubes to which are added 9, 8, 7, 6, 5, 4, 3, 2, 1 and 0 cc of faintly alkalized water. They should be tightly corked and preferably sealed with paraffin. Their color will be retained for several months if kept in the dark, but they will rapidly lose color if exposed to the sunlight. These tubes represent from 10 to 100 per cent standards. Satisfactory comparisons can be made with them by holding the tubes directly against a sheet of white paper or other white background.



## REVIEWS

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Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,  
Richmond, Va

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### *How I Came to Be* \*

A BOOK on human embryology, written for the layman who is curious to know the mysteries of the origin and development of the human fetus. The author has added interest by couching the discussion in the form of an autobiographic story, the unborn babe telling in his own way his experiences from day to day, from the time of his first extrusion from the ovary.

It is a difficult subject well handled. Difficult, not from any aspect of prudery, but because even with a good instructor and an intelligent student there are many stages in embryologic development that are not readily visualized. However, while the reader may not be able to follow consecutive development of the various organs step by step, he will easily acquire from the book a fairly good knowledge of what is going on during embryologic development.

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### *Statistical Methods for Research Workers* †

THIS is the fifth volume of a series of biologic monographs and manuals the purpose of which is to present an authoritative summary, review and discussion of various fields of biologic investigation.

In many researches the analytic survey of large masses of data by statistical methods is essential and the purpose of the present volume is to present in a relatively simple and clear way the methods whereby such statistical problems may be attacked.

To those required or desiring to use applied mathematics in the analysis of statistical data this volume should be of inestimable value as the subject, necessarily complicated, is presented in a series of clear and logical steps accompanied by numerous tables illustrated and facilitating their practical application.

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### *Food Infections and Food Intoxications* ‡

THE purpose of this book is to present in a single volume an authoritative survey of the subject of poisoning and intoxication from food. To this end a large amount of clinical and experimental data scattered throughout the literature has been consulted and discussed.

The resultant volume, apparently the first of its kind, furnishes a very compact, comprehensive, and clear presentation of this very important subject.

The book is divided into three sections, the first, Infections from Food (58 pages),

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\*How I Came to Be. The Autobiography of an Unborn Infant. By Armenouhie T Lamson. Pp 179. Macmillan. New York 1926.

†Statistical Methods for Research Workers. By R. A. Fisher. Chief Statistician Rothamsted Experimental Station. Cloth. Second edition. 269 pages. 11 figures. numerous tables. Oliver and Boyd. Edinburgh.

‡Food Infections and Food Intoxications. By S. R. Damon. Associate Professor of Bacteriology, Johns Hopkins University School of Hygiene. Cloth. 266 pages. 33 figures and 1 plate. Williams and Wilkins Co. Baltimore.

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NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

discussing paratyphoid in actions from food tuberculosis from milk and meat, undulant fever from milk, septic sore throat from milk and retinomyces. Whenever possible the phases of etiology, symptomatology, diagnosis treatment and prophylaxis are presented in detail. Under diagnosis, acceptable laboratory methods are described.

The experimental evidence in each type of food poisoning receives due consideration. Section II (91 pages) is devoted to a discussion of intoxications from Food, Botulism mushroom poisoning, and fish and shell fish poisoning each being discussed in accordance with the plan above outlined.

In Section III (87 pages) zoonotic infections acquired through food are considered, animal parasites, trichinosis trematosis helminth infestation, and other parasites.

The book is deserving of and should receive a warm welcome from physicians and laboratory and public health workers. It is a volume to be purchased rather than borrowed.

### *Gonococcal Urethritis in the Male\**

SO VOLUMINOUS has the literature upon disease become that while the textbook, as ordinarily understood is needed as a repository for known facts and a comprehensive source of reference, there is a growing and in many ways commendable, tendency to resort to the monograph for comprehensive discussions of a single subject or some single phase thereof.

The author of the present volume begins his preface by expressly disclaiming any intention of presenting it as a textbook and classifies it as 'a simple story simply told'. His intention is to place before his readers the lessons drawn from his experience in the treatment of gonorrhea in the male and that he has had ample experience, and that it has been profitably digested a perusal of the volume well demonstrates.

He maintains, and with justice in the reviewer's opinion, that gonorrhea is a disease which in the minds of all too many is but little understood, its pathology but ill appreciated, and the manner of its treatment too many times haphazard.

Dr. Pelouze then, in a clear and simple manner discusses the subject from all these angles, with the courage of his convictions and certainly from the standpoint of experience.

In the reviewer's opinion this book could well be read with profit by the practitioner at large and serve as a starting point, at least for an intelligent method of treatment. It can be highly recommended for the use of those to whom it was written.

### *Handbook of Histology†*

THE purpose of this little volume to present a short, concise description of tissues and organs with illustrations prepared from class specimens has been very successfully accomplished. The text is well written and the illustrations carefully selected and prepared. The book should serve a useful purpose.

### *Aluminum Compounds in Food‡*

CONSIDERING the rather bitter controversy which has been and still is being waged by various commercial interests, chiefly regarding the use of baking powders containing aluminum and aluminum cooking utensils, and the many obviously prejudiced publications which have appeared, it is refreshing to review this book by Dr. E. E. Smith which presents a complete digest of the literature upon 'Aluminum Compounds in Foods' written with

*Gonococcal Urethritis in the Male For Practitioners* By P. S. Pelouze M.D. Associate in Urology and Assistant Genito Urinary Surgeon at the University of Pennsylvania. 35 pages 78 illustrations. Cloth Philadelphia and London W. B. Saunders Co. 10-S.

*A Handbook of Histology* By A. McL. Watson Lecturer on Histology University of Glasgow. Cloth 207 pages 50 figures 1 colored plate W. Wood and Co. New York.

*Aluminum Compounds in Food* By Ernest Elsworth Smith Ph.D. M.D. Cloth Pp 38 Paul B. Hoeber Inc. 78 5th Ave. New York 1023.

the evident purpose of presenting all the known facts. In all except the final chapter in which he endeavors to draw conclusions from this mass of data, Dr Smith presents the findings and opinions of the various investigators whose work he reviews in an entirely impartial way, often in the authors' words.

The methods of estimating the minute amounts of aluminum present in various animal tissues have been admittedly very unsatisfactory, and the reviewer believes that the figures given in the literature for the somewhat larger amounts of aluminum naturally occurring in foods should be accepted with some reservations.

Since the presence and effects of aluminum compounds in food are of such scientific interest at the present time, it is helpful to have in a single volume all available data on the subject, including a comprehensive résumé of the report of the Referee Board of the U S Department of Agriculture, which has not been readily accessible owing to the fact that the report was never printed.

Chapter II gives the natural occurrence of aluminum in food, Chapter III, added aluminum compounds in food, Chapter IV, history and research, 1828-1897, Chapter V, researches of E E Smith, Chapter VI, the influence of aluminum compounds on the nutrition and health of man, Chapter VII, litigations relative to baking powders containing salts of aluminum, Chapter VIII, the relation of aluminum compounds on unicellular animal life and on the isolated cells and tissues of higher animals, Chapter X, the solubility in the gastrointestinal tract of aluminum compounds of baking powder residues, Chapter XI, the action in the alimentary tract of food prepared with sodium aluminum sulphate baking powders, Chapter XII, the question of the absorption of aluminum, Chapter XIII, the effects of aluminum compounds when administered subcutaneously or intravenously, Chapter XIV, experimental observations upon the influence of food prepared with baking powder containing sodium aluminum sulphate on the growth and well being of animals, and Chapter XV, discussion.

The book is an interesting, readable narrative on the subject of aluminum in foods. It is complete, authoritative, and impartial, and will be found very useful to physicians, chemists and others interested in this subject.

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## EDITORIALS

### The Essential Nature of Pernicious Anemia

**I**S PERNICIOUS anemia due to an increased destruction of red blood corpuscles or does it result from a disturbance of blood formation? Diverse views have been held by hematologists for the past fifty years. Most workers have considered the evidence for increased blood destruction conclusive, the great excess of bile pigments and other end products of hemoglobin metabolism in the blood, tissues and secretions during the clinically active stage of the disease being the principle reason for considering the disease as primarily hemolytic in nature. Some unknown hemolytic agent has been generally accepted as the cause of the disease.

The most characteristic finding at autopsy is the replacement of bone marrow ordinarily filled with fat by hyperplastic red marrow. This marked hyperplasia has been usually interpreted as an attempt to compensate by increased blood formation for the increased blood destruction. The inevitably fatal outcome thus represented the exhaustion of the bone marrow to a point at which it could no longer produce red cells.

Another constant finding at autopsy is the deposit of hemosiderin in the liver, spleen, and kidneys supposedly due to the increased blood destruction. The characteristic clinical and pathologic findings have been correlated to visualize a disease in which there is primarily excessive blood destruction from some toxic agent, followed by a compensatory hyperplasia of the myeloid tissue. If this view were correct, during the remissions of the disease the bone marrow should be forming more cells than during a relapse.

On the other hand some workers have considered the disease as primarily one of the bone marrow. This was the view of Cohenheim who first described in 1876 the characteristic bone marrow findings at autopsy. He considered the disease as due to a disturbance of blood formation. The evidence for increased blood destruction has seemed so overwhelming few have accepted this view. Whipple in 1922<sup>1</sup> suggested that the essential nature of pernicious anemia was a deficiency in stroma formation. He concluded that "pernicious anemia should be looked on as a scarcity of stroma building materials or a disease of the stroma forming cells of the marrow which limits the output of red cell framework."

Until recently the bone marrow was studied only from autopsy material. The findings in the terminal stage were considered as typical of all stages of the disease. A study of the bone marrow changes in material obtained by bone marrow puncture, a procedure introduced by Ghedini in 1908,<sup>2</sup> has yielded information of the greatest value concerning the nature of pernicious anemia. Specimens were first obtained from the bone marrow of patients suffering from pernicious anemia in different stages of the disease by Zadek.<sup>3</sup> He found that the bone marrow returns to normal with a remission. He also noted that with the characteristic megalocytosis of a relapse there is a great increase in the megablasts of the bone marrow.

Using the technic of marrow puncture Peabody<sup>4</sup> also greatly extended our knowledge of the changes in the bone marrow during the course of the disease. He showed that the characteristic hyperplasia of the megablasts is the more striking the more active the disease and the more profound the relapse. The hyperplasia of the relapse becomes less evident as clinical improvement begins. During a remission only the normal bone marrow reaction may be found.

This development of a megaloblastic hyperplasia in severe relapses and disappearance with the remission is the strongest evidence against a hemolytic disease. It cannot be correlated with the view that increased blood destruction is the primary change followed by a compensatory bone marrow reaction. The findings can be explained only on the supposition that the essential pathologic lesion is in the bone marrow. Peabody concluded that "the course of the anemia would thus appear to be an abnormal type of cell growth consisting in a development of the primitive megablast and a failure of differentiation into more mature cells that normally get into the blood." The presence of the excess amount of bilirubin in the blood, tissues, and secretions must still be explained. This probably represents, as Peabody suggests, an excess of pigment over what the bone marrow can use in building red cells in the

active stage of the disease which in fact represents a period in the disease when very few cells are being put out into the circulation. The exceedingly small number of vital staining cells at this period also is corroborative evidence that very few cells are being formed.

The localization of the essential lesion of the disease in the bone marrow does not solve the problem of the etiology. Some toxin of the type long supposed to be the hemolytic agent might conceivably so affect the bone marrow. A lack of stroma building material as suggested by Whipple might produce the same pathologic picture. The results attained by treatment with liver and liver extract throw some light on the question. The liver extract which is equally as potent as liver is free of protein, carbohydrate, non lipoids and all known vitamins. It has the chemical characteristics of a nitrogenous base or a polypeptide. Its action is due to a specific maturating effect on the megalo blasts.<sup>3</sup>

We must look on pernicious anemia as due primarily to an inability of the bone marrow to mature red cells resulting from the lack of some specific substance and thus classify it among the deficiency diseases. Tissues other than the liver, such as the kidney, can furnish the specific substance, so the deficiency is not necessarily an hepatic one. It is more likely due to a failure of absorption or an elaboration in the intestinal tract of the specific maturating substance, perhaps resulting from a long existent achlorhydria, a constant feature of the disease.

## REFERENCES

- <sup>1</sup>Whipple G H Arch Int Med 1922 xix 711  
<sup>2</sup>Ghedin, G Wien Med Wchnschr 1911 xiv 284  
<sup>3</sup>Ludek J Ztschr f klin Med 1902 lxxv 66  
<sup>4</sup>Peabody F W Am Jour Path 1927 iii 179  
<sup>5</sup>Cohn, E J Minot, G R Alles G A and Salter W T Jour Biol Chem 1928 lxxviii, 325

—P L H

### *Experimental Studies in Chronic Lesions of the Lung*

SOME of the recent bacteriologic studies in the production of chronic abscess of the lung have revealed information of great interest to the laboratory worker as well as to the clinician. Louis Herrmann, working under Elliott Cutler, in the Laboratory of Surgical Research at Western Reserve has modified the original Cutler Sehneter method and he has been able to produce, at will, chronic abscess of the lung for study over periods of over one hundred days. This modified procedure is dependent upon the interesting fact that anaerobic organisms with spirochetes and fusiform bacilli obtained from scrapings of pyorrheal sacs are evidently important factors in the chronicity of lung lesions. Some of these abscesses of the lung have been under study for the past year, and these present all of the clinical and pathologic earmarks of typical chronic human lesions. Vein segments or blood clots impregnated with pyorrheal exudate were introduced intravenously and the infected emboli lodging in the lung produced the pulmonary abscesses. Thus typical pure lesions uncontaminated by bronchial manipulation, were secured for study.

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That the spirochetes are only occasionally the chief factor in these conditions is clinically evident from the rarity of the spectacular therapeutic response of these cases to arsphenamine therapy. In this connection, it is interesting to note that in silver impregnation studies of these tissues of experimental lung abscess, the spirochetes were found in advance of the pyogenic process in the lung parenchyma. The further running down of the anaerobes that are chiefly responsible for the chronicity is being pursued. The reproduction of abscess in fresh dogs from abscesses in previous dogs and from the various reclaimed and isolated organisms from experimental lesions has incriminated the anaerobes especially, as the chief factors in chronicity.

This experimental work emphasizes the importance of careful clinical laboratory studies and especially bacteriologic examinations of the sputum or expectorated exudate in cases of pulmonary abscess, ganglionic bronchiectasis and chronic bronchitis.

## REFERENCES

- Cutler, Elliott C. Reunion Massachusetts General Hospital Alumni, February, 1928, Boston.  
 Herrmann, Louis G. Reunion Peter Bent Brigham Hospital Alumni, April, 1928, Boston.  
 Cleveland Meeting of the Southern Clinical Club, May, 1928.  
 Herrmann, L. G. and Cutler, E. C. *Proc Soc Exper Biol and Med* 1928, **xxvi**, 28.  
 Holloway, J. W., Schlueter, S. A., and Cutler, E. C. *Ann Surg* 1927 **lxxxvi**, 165.  
 Kline, B. S. *Jour Infect Dis* 1923 **xxxii**, 481.  
 Smith, D. T. *Arch Surg*, 1927 **xiv**, 231.  
 Crowe, S. J. and Scarff, J. E. *Arch Surg* 1928 **xvi**, 176.  
 Allen, D. S. *Arch Surg* 1928 **xvi**, 179.  
 Ochsner, A. and Nesbitt, W. *Am Jour Med Sc* 1928 **clxxv**, 170.

—G H



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## News Items

The Service Bureau of the American Society of Clinical Pathologists has the following positions open for clinical pathologists

A large insurance company desires a physician for correspondence work in connection with longevity service. This position does not involve any laboratory work but requires a knowledge of clinical pathology.

Several positions are open requiring doctors with a knowledge of clinical microscopy, serology, bacteriology, physiologic chemistry, postmortem technique and tissue pathology, salary range, \$3800 to \$5000.

There is also a very excellent opening in an Eastern Hospital as Director of Laboratories requiring a man highly recommended and of unquestioned ability.

Any member of the Society interested in any of these positions, communicate immediately with the Secretary, Dr H J Corper, Children's Hospital, Denver, Colorado.

The Registry of Technicians under the auspices of the American Society of Clinical Pathologists is being carried on in the Office of the Secretary, Children's Hospital, Denver, Colorado.

Registration blanks will be sent on request.

The Registry also includes an employment service, and invites physicians seeking competent technicians to communicate with this department.

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## CLINICAL AND EXPERIMENTAL

### THE EFFECT OF ETHER ANESTHESIA AND SHOCK ON THE CALCIUM OF THE BLOOD\*

BY WILLIAM C EMERSON MD ROCHESTER N Y

THE fact that calcium salts or parathyroid hormone when administered to jaundiced patients reduces the toxicity of the bile in the blood or tissues has never been observed. If calcium is of aid only in shortening the coagulation time of the blood we would not expect an increase in the serum calcium during jaundice.

However King and Stewart<sup>1</sup> report that in jaundiced dogs there is a 17 to 20 per cent increase in the serum calcium. King Bigelow and Pearce state that there is a higher serum calcium content in the blood of jaundiced dogs than in the blood of normal dogs. Davidson and Emerson<sup>2</sup> have found an increase in the serum calcium following the injection of whole bile into the femoral vein of a dog.

Lee and Vincent<sup>3</sup> have reduced the coagulation time of the blood in jaundiced patients, in vivo as well as in vitro, by the addition of calcium salts to the blood. Kirl and King report that in a series of seven cases of jaundiced patients the diffusible calcium is below normal.

It occurred to us that there must be some explanation for this apparent conflict. Three authors reported an increase in the serum calcium in jaundice and others reported that the diffusible calcium was lowered in jaundice and that the addition of calcium salts to the blood decreased the coagulation time. That anesthesia or shock might have some effect on the serum calcium and might explain the results obtained by the three authors was considered.

King and Stewart<sup>1</sup> do not state whether blood was obtained for the determination of serum calcium before or after anesthesia. Kirk Bigelow

\*From the Department of Surgery, University of Rochester School of Medicine and Dentistry, Rochester, New York.

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and Pearce report that a week after the ligation of the common duct, the dogs were bled to death under ether anesthesia. Davidson and Emerson<sup>3</sup> do not state in their report whether blood was obtained for the determination of serum calcium before or after anesthesia but it is known that it was obtained following ether anesthesia. The blood for the normal serum calcium determinations was obtained before ether anesthesia.

Binet and Blanchetiere<sup>4</sup> state that in their research work under chloral the venous blood contains more calcium than the arterial and that asphyxia is accompanied by hypercalcemia preceded by a transient hypocalcemia.

Bokelmann and Boeck<sup>5</sup> have shown that the total calcium content of the blood from the umbilical vein of the fetus is greater than the total calcium content of the blood from the umbilical artery.

Van Slyke, Austin and Cullen<sup>6</sup> in experiments on dogs have shown that the  $P_H$  and the alkaline reserve of the blood fell markedly following ether anesthesia. They also report in one experiment a decrease in the oxygen content of the blood from 9.70 before ether anesthesia to 8.55 after ether anesthesia.

The increase in the acidity of the blood, the decrease in the alkaline reserve and the oxygen content of the blood following ether anesthesia would indicate a lowering of the  $CO_2$  carrying capacity of the blood and a certain amount of asphyxia.

Those familiar with anesthesia either on patients or dogs have observed periods of cyanosis and slowing of the respiratory rate, indicative of asphyxia, during the administration of any general anesthetic.

Cantariow, Dodak and Gordon<sup>7</sup> state that alteration of the hydrogen-ion concentration of the blood will cause some disturbance in the functional availability and utilization of the calcium.

Taylor and Caven<sup>10</sup> believe that anesthesia has no effect on the serum calcium.

#### METHOD

Ten normal dogs, varying in weight from 10 to 12 kilos were obtained. They were placed on a mixed feed consisting of meat, bread, bone, fruit, vegetables, and pasty all of which were ground and mixed thoroughly. The dogs received no food on the day of the experiment.

Blood was obtained from the jugular vein in all the experiments and the normal serum calcium determined according to the method of Clark and Collip.<sup>11</sup> The animals were anesthetized by the cone method. A large metal cone with a hole in the closed end was used. Gauze soaked with ether was placed in the closed end, and ether added from time to time through the hole in the cone. The animals in each experiment were kept in the second stage of anesthesia for an hour. Blood was then taken and the serum calcium determined.

A week later each animal was given a second anesthetic, blood being taken before and after anesthesia and the serum calcium determined.

A week after the second anesthetic, five of the dogs were operated on under ether anesthesia. In each case a major operation was performed i.e.

TABLE I

DOG NO	WT OF DOG KG	MG SERUM CALCIUM PER 100 CC										OPERATION
		BEFORE ANESTHESIA	AFTER ANESTHESIA	CHANGE AFTER ANESTHESIA	BEFORE SECOND ANESTHESIA	AFTER SECOND ANESTHESIA	CHANGE AFTER SECOND ANESTHESIA	BEFORE ANESTHESIA AND OPERATION	AFTER ANESTHESIA AND OPERATION	CHANGE AFTER ANESTHESIA		
1	123	113	136	23+	119	140	21+	121	131	10	Cholecystectomy	
2	110	115	125	10+	117	136	19+	117	128	11	Cholecystectomy	
3	120	120	132	12+	126	144	18	120	130	10+	Cholecystectomy	
4	100	124	141	16+	122	130	8	121	128	7	Cholecystectomy	
5	118	125	141	16+	121	131	10+	121	131	10	Cholecystectomy	
6	122	114	135	21+	119	138	19	124	134	10	Cholecystectomy	
7	100	124	141	17+	122	143	21+	122	139	17	Cholecystectomy	
8	114	110	134	24+	114	126	12	114	126	12	Cholecystectomy	
9	100	106	125	19+	107	139	32+	107	139	32	Cholecystectomy	
10	118	121	144	23+	124	153	29	124	153	29	Cholecystectomy	
Average		110	137	21+	118	138	20+	120	131	11+		

quiring the opening of the peritoneal cavity. Blood for serum calcium determinations was taken before and after the operation and anesthetic.

Five more normal dogs, varying in weight from 12 to 20 kilos, were obtained. They were placed on the mixed feed and were given no food the day of the experiment.

The hole in the cone was plugged up. The cone was then placed over the dog's nose and mouth tightly and for a period of five minutes each dog was asphyxiated. The animals received some air around the edge of the cone but only a little. Blood for serum calcium was taken before and after asphyxia and the calcium content determined.

A week later each animal was anesthetized with ether and hyperventilation produced by the addition of  $\text{CO}_2$  through a rubber tube placed in the hole of the cone. The dog's respiratory rate was increased to fifty per minute. The animals were kept in the second stage of anesthesia for an hour. Blood for serum calcium determinations was taken before and after anesthesia.

A week later each animal was operated on under local anesthesia, 1 per cent novocaine being used and in each instance a major operation performed necessitating the opening of the peritoneal cavity. Blood was taken before and after the operation and the serum calcium determined.

### RESULTS

The serum calcium of the blood was determined in ten dogs before and after ether anesthesia. A week later the serum calcium in the blood of the same dogs was determined before and after ether anesthesia. A week later five of the dogs were operated upon under ether anesthesia and the serum calcium of the blood determined before and after the operation. These results are summarized in Table I.

Five other normal dogs were obtained and the serum calcium of the blood determined before and after asphyxia. These results are summarized in Table II.

TABLE II

DOG NO	WT OF DOG	BEFORE ASPHYXIA	AFTER ASPHYXIA	CHANGE AFTER ASPHYXIA
		SERUM CALCIUM PER 100 C C		
	KG	MG	MG	MG
11	17.8	11.0	14.3	3.3+
12	20.2	12.8	15.8	3.0+
13	18.4	12.0	14.9	2.9+
14	12.8	12.0	13.9	1.9+
15	16.3	11.5	12.5	1.0+
Average		11.8	14.2	2.4+

A week later these dogs were anesthetized with ether and hyperventilation produced by the addition of  $\text{CO}_2$  to the ether mixture. The serum calcium of the blood was determined before and after anesthesia with hyperventilation. These results are summarized in Table III.

TABLE III

DOG NO	WT OF DOG KG	BEFORE ANESTHESIA PLUS CO <sub>2</sub>	AFTER ANESTHESIA PLUS CO <sub>2</sub>	CHANGE AFTER ANES- THESIA PLUS CO
		SERUM CALCIUM PER 100 C.C.		
		MG	MG	MG
11	17.8	11.5	10.6	0.9-
12	20.2	12.4	11.3	1.1-
13	18.4	12.4	11.6	0.8-
14	12.8	12.2	11.4	0.8-
15	16.3	11.8	11.8	0.0
Average		11.6	11.3	0.7-

TABLE IV

DOG NO	WT OF DOG	BEFORE OPERATION LOCAL ANESTHESIA	AFTER OPERATION LOCAL ANESTHESIA	CHANGE AFTER OPERATION LOCAL ANESTHESIA	OPERATION
		SERUM CALCIUM PER 100 CC			
		MG	MG	MG	
11	17.8	11.5	10.4	1.1-	Cholecystectomy splenectomy Appendectomy Cholecystectomy Lateral anas- tomosis of the small intestine
12	20.2	12.6	10.6	2.0-	
13	18.4	11.7	11.4	0.3-	
14	12.8	12.4	12.2	0.2-	
15	16.3	11.4	12.0	0.6+	
Average		11.7	11.3	0.6	

A week later these five dogs were operated upon under local anesthesia. The serum calcium of the blood was determined before and after operation. These results are summarized in Table IV.

## DISCUSSION

The rise in serum calcium following anesthesia, an average of 2.1 mg per 100 c.c. compares very well with the rise in serum calcium following the injection of whole bile into the femoral vein as reported by Davidson and Emerson. They report in a series of ten dogs an average rise of 2.8 mg. of serum calcium per 100 c.c. following the injection of whole bile.

The rise of 2.1 mg per 100 c.c. above the normal of 11.6 mg per 100 c.c. equals 18 per cent in the serum calcium following ether anesthesia. This agrees with King and Stewart's<sup>1</sup> findings of a 17 to 20 per cent increase in the serum calcium of jaundiced dogs. This reported increase is not due to the jaundice but to the anesthesia.

The average rise of only 1.1 mg per 100 c.c. of serum calcium following a general operation under ether anesthesia and the slight fall an average of 0.6 mg per 100 c.c. following a major operation, under local anesthesia would clearly indicate that shock has little or no effect on the serum calcium content of the blood.

The increase in serum calcium following asphyxia, an average of 2.4 mg per 100 c.c. as compared to the average of 2.1 mg per 100 c.c. following

anesthesia shows that the rise of serum calcium following anesthesia is due to the asphyxia. This is even more markedly demonstrated by the fall in serum calcium, an average of 0.7 mg per 100 cc following anesthesia in which there had been hyperventilation.

Taylor and Caven<sup>10</sup> probably kept their animals under a very light anesthesia and asphyxia was not produced.

These results would indicate that a certain amount of asphyxia during a general anesthetic is of value in as much as the serum calcium is increased, an average of 18 per cent which would tend to decrease the coagulation time of the blood and prevent hemorrhage.

#### CONCLUSIONS

1 There is a definite increase, 18 per cent, in the serum calcium of the blood following ether anesthesia.

2 There is an increase, 20 per cent, in the serum calcium of the blood following asphyxia.

3 There is a slight decrease in the serum calcium of the blood following anesthesia in which there is hyperventilation.

4 A slight amount of asphyxia during ether anesthesia is of value as it tends to raise the serum calcium content of the blood and thus shorten the coagulation time.

5 Shock has no effect upon the serum calcium of the blood.

#### REFERENCES

- <sup>1</sup>King, J. H., and Stewart, H. A. Effect of Injection of Bile on the Circulation, *Exper Med*, 1909, **1**, 673-685.
- <sup>2</sup>King, J. H., Bigelow, J. E., and Peirce, L. Experimental Obstructive Jaundice, *Exper Med*, 1911, **14**, 159-178.
- <sup>3</sup>Davidson, E. C., and Emerson, W. C. The Influence of the Liver Upon the Toxicity of the Bile, *Arch Surg*, 1927, **11**, 57-65.
- <sup>4</sup>Lee, R. I., and Vincent, B. The Relation of Calcium to the Delayed Coagulation of the Blood in Obstructive Jaundice, *Arch Int Med*, 1915, **11**, 59-66.
- Kirk, P. L., and King, C. G. Calcium Distribution in the Blood, *Jour Lab and Clin Med*, 1926, **11**, 928-935.
- <sup>5</sup>Binet, L., and Blanchetiere, A. Calcium Content in Arterial and Venous Blood, Increased Blood Calcium in Asphyxia, *Compt rend Soc de biol*, 1925, **11**, 511-512.
- <sup>6</sup>Bokelmann, O., and Bock, A. Calcium Ion Concentration in Pregnancy and Its Relation to Convulsions, *Klin Wchenschr*, 1927, **11**, 2427.
- <sup>7</sup>Van Slyke, D. D., Austin, J. H., and Cullen, G. E. The Effect of Ether Anesthesia on the Acid Base Balance of the Blood, *Jour Biol Chem*, 1922, **11**, 277-292.
- <sup>8</sup>Cantrow, A., Dodak, S. M., and Gordon, B. Calcium Studies in Jaundice, *Arch Int Med*, 1927, **11**, 129-139.
- <sup>9</sup>Taylor, N. B., and Caven, W. R. Observations Upon Serum Calcium after Adrenalectomy, *Am Jour Physiol*, 1927, **11**, 511-512.
- <sup>10</sup>Clark, E. P., and Collip, J. B. Tisdall Method for the Determination of Serum Calcium with a Suggested Modification, *Jour Biol Chem*, 1925, **11**, 641-646.

# THE PHYSIOLOGIC BEHAVIOR OF GLYCERYL TRIMARGARATE\*

By DOROTHY L. R. MOORE, M.S., MARIAN BRUCKENRIDGE, M.S., AND  
HILDA CROLL KOSLER, PH.D., URBANA, ILLINOIS

IN AN attempt to solve some of the problems occasioned by the restrictions of diabetic diets, Kahn and Nolan<sup>1</sup> about 1923 introduced a synthetic odd carbon atom fat glyceryl trimargarate or intarvin. On the basis of the beta oxidation theory of fat metabolism, Kahn anticipated that the diabetic patient would be able to catabolize glyceryl trimargarate without the excretion of acetone bodies since theoretically this synthetic fat might be expected to be burned in the body to a derivative of propionic rather than of butyric acid, from which the acetone bodies are derived. Following the description of this fat, various investigations have been conducted on the physiologic effect and the practical value of intarvin in the treatment of diabetic patients. From a review of the reports of these investigations it is evident that opinions vary concerning the influence of intarvin on the formation of acid substances in diabetic and normal subjects. The use of intarvin in the dietary treatment of diabetes seems to present practical difficulties which have not been solved successfully. The experiments here reported were undertaken to add to the existing information on the subject by studying the physiologic effect of intarvin on the condition of ketosis produced experimentally in normal individuals.

## EXPERIMENTAL PROCEDURE

Five experiments were conducted on three individuals, healthy young women, members of the faculty of the Department of Home Economics, two of these women served twice as subjects the third once. Their daily energy requirements for sedentary occupations from 1750 to 2100 calories were supposedly provided for by the diets which contained approximately 2000 calories. The subjects were given basal diets low in carbohydrate and fairly high in protein and fat, in order to produce acidosis. 100 or 130 grams of fat were added daily to the basal diets. Butter was the fat used during the first two or three days the preliminary period during which time ketosis was produced in every instance. This period was followed immediately by the three experimental days at which time intarvin was substituted for the butter in order to study the effect of this odd carbon synthetic fat on the acidosis produced by the even carbon naturally occurring fats.

During Experiments I, II, and III the subjects were given a basal diet

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(The menus for the three diets are described in the master's thesis of D. L. R. Moore, University of Illinois, 1933. About one third of the butter and intarvin supply for the day was given with each of the three meals although 10 grams more of the fat were given with dinner (the largest meal) than with breakfast or lunch.)



consisting of 100.5 grams of protein, 75.5 grams of fat, and 25 grams of carbohydrate, to which were added 118 grams\* of butter in the preliminary period and 100 grams of intarvin in the experimental period. The basal diet† for Experiments IV and V consisted of 80 grams of protein, 50 grams of fat, and 25 grams of carbohydrate, to which were added 130 grams of fat in the form of butter or intarvin. These reductions in the protein and fat content of the basal diets and the increases in added butter fat and intarvin were made in order to have a larger proportion of the fat of the diet in the form of the two fats under investigation, thus making possible a sharper contrast between any effects which they might have on the condition of acidosis.

These fats were added to the diet in various ways. Some of the butter was eaten separately in small lumps, the rest being mixed with the food or melted in hot coffee. Some difficulty was encountered in finding a satisfactory way of ingesting the intarvin‡. This fat has a white creamy color, a tallow-petroleum-like odor and an unpleasant tallowy taste which persists for some time. It is very bulky (one tablespoonful weighing six grams) and it has such a high melting point that it tends to solidify in the mouth when taken melted. An attempt was made to conceal its flavor by using it mixed with other ingredients in recipes. Bran muffins flavored with ginger, stewed tomatoes flavored with onions and cloves, and scrambled eggs were the foods tried, but the products containing intarvin were very unpalatable. Intarvin was also put into capsules, but the large quantity of fat required the ingestion of too many capsules to be considered practicable. An attempt to take the fat in the form of large pills was also unsatisfactory, because this method necessitated the ingestion of too much water with the meals. The method finally found least objectionable, and the one which was used in the experimental periods, was that of melting the intarvin and drinking it mixed with hot black coffee, which seemed to mask some of the unpleasant properties of the fat. Difficulty in ingestion of this fat because of these properties was noted in most of the reports of investigations on intarvin.

Quantitative urine analyses were made on twenty-four-hour urine specimens, collected in all cases during the first and last days of both the preliminary and experimental periods, and in Experiments II and III during the day preceding the preliminary period, when the subjects were on a normal mixed diet. Determinations were made for creatinin (Fohn's method<sup>3</sup>), organic acids (Van Slyke and Palmer<sup>4</sup>), ammonia (Fohn and Bell), total titrable acidity (Fohn<sup>6</sup>) and total acetone bodies (Van Slyke<sup>7</sup>). The relation of these substances to the acid-base balance of the body is well known.

In Experiment I, the amount of fat absorbed by the body was estimated by quantitative determination of fat in the feces during both the preliminary and experimental periods (Saxon method<sup>8</sup>).

\*Equivalent to 100 grams of fat since butter contains 85 per cent of fat.

†See footnote on preceding page.

‡The intarvin was secured from the Bergmann Laboratories Intarvin Company, Inc. College Point, New York.

TABLE I  
RESULTS OF URINE ANALYSES IN EXPERIMENTS I TO V

DATE	DIET	VOLUME CC	SP GR	REACTION	CREAT ININ MG	AMMONIA GM	ACETONE BODIFS GM	ORGANIC ACIDS CC 0.1 N	TOTAL ACIDITY CC 0.1 N
1926	Subject I				Experiment I				
May 25	Basal diet and butter	900	1.028	acid	1213	0.17	1.17	634	31.
27	Basal diet and butter	1850	1.020	acid	1103	0.81	3.27	980	505
28	Basal diet and intarvin	3680	1.011	acid	1309	1.07	2.79	982	466
30	Basal diet and intarvin	1600	1.023	acid	1168	0.93	3.66	1069	461
	Subject I				Experiment II				
Oct 24	Normal	760	1.022	acid	1145	0.37	0.09	385	272
25	Basal diet and butter	1030	1.025	acid	1256	0.41	0.34	475	271
27	Basal diet and butter	1640	1.010	acid	1240	0.77	1.80	728	502
28	Basal diet and intarvin	1000	1.031	acid	1265	0.71	1.63	937	471
30	Basal diet and intarvin	1000	1.026	acid	1000	0.90	2.47	1006	459
	Subject II				Experiment III				
Oct 24	Normal	1730	1.012	acid	983	0.32	None	411	134
25	Basal diet and butter	1550	1.019	acid	1078	0.35	0.49	483	247
27	Basal diet and butter	1170	1.020	acid	1023	0.70	1.06	519	480
28	Basal diet and intarvin	1280	1.020	acid	1091	0.64	0.88	500	520
30	Basal diet and intarvin	1350	1.022	acid	963	0.71	1.71	859	441
1927	Subject II				Experiment IV				
April 5	Basal diet and butter	1200	1.017	acid	1023	0.39	0.28	453	163
6	Basal diet and butter	1220	1.020	acid	1030	0.43	1.02	580	364
7	Basal diet and intarvin	1100	1.020	acid	947	0.67	1.47	723	460
9	Basal diet and intarvin	1550	1.022	acid	1040	0.78	1.69	923	496
	Subject III				Experiment V				
April 5	Basal diet and butter	1000	1.021	acid	1125	0.44	1.04	608	256
6	Basal diet and butter	1250	1.020	acid	1122	0.78	3.60	985	419
7	Basal diet and intarvin	1380	1.021	acid	1145	0.84	3.28	859	448
9	Basal diet and intarvin	880	1.030	acid	1009	0.96	3.54	1095	377

consisting of 100.5 grams of protein, 75.5 grams of fat, and 25 grams of carbohydrate, to which were added 118 grams of butter in the preliminary period and 100 grams of intarvin in the experimental period. The basal diet† for Experiments IV and V consisted of 80 grams of protein, 50 grams of fat, and 25 grams of carbohydrate, to which were added 130 grams of fat in the form of butter or intarvin. These reductions in the protein and fat content of the basal diets and the increases in added butter fat and intarvin were made in order to have a larger proportion of the fat of the diet in the form of the two fats under investigation, thus making possible a sharper contrast between any effects which they might have on the condition of acidosis.

These fats were added to the diet in various ways. Some of the butter was eaten separately in small lumps, the rest being mixed with the food or melted in hot coffee. Some difficulty was encountered in finding a satisfactory way of ingesting the intarvin‡. This fat has a white creamy color, a tallow-petroleum-like odor and an unpleasant tallowy taste which persists for some time. It is very bulky (one tablespoonful weighing six grams) and it has such a high melting point that it tends to solidify in the mouth when taken melted. An attempt was made to conceal its flavor by using it mixed with other ingredients in recipes. Bran muffins flavored with ginger, stewed tomatoes flavored with onions and cloves, and scrambled eggs were the foods tried, but the products containing intarvin were very unpalatable. Intarvin was also put into capsules, but the large quantity of fat required the ingestion of too many capsules to be considered practicable. An attempt to take the fat in the form of large pills was also unsatisfactory, because this method necessitated the ingestion of too much water with the meals. The method finally found least objectionable, and the one which was used in the experimental periods, was that of melting the intarvin and drinking it mixed with hot black coffee, which seemed to mask some of the unpleasant properties of the fat. Difficulty in ingestion of this fat because of these properties was noted in most of the reports of investigations on intarvin.

Quantitative urine analyses were made on twenty-four-hour urine specimens, collected in all cases during the first and last days of both the preliminary and experimental periods, and in Experiments II and III during the day preceding the preliminary period, when the subjects were on a normal mixed diet. Determinations were made for creatinin (Folin's method<sup>3</sup>), organic acids (Van Slyke and Palmer<sup>4</sup>), ammonia (Folin and Bell), total titrable acidity (Folin<sup>5</sup>), and total acetone bodies (Van Slyke<sup>6</sup>). The relation of these substances to the acid-base balance of the body is well known.

In Experiment I, the amount of fat absorbed by the body was estimated by quantitative determination of fat in the feces during both the preliminary and experimental periods (Saxon method<sup>8</sup>).

\*Equivalent to 100 grams of fat since butter contains 83 per cent of fat.

†See footnote on preceding page.

‡The intarvin was secured from the Bergmann Laboratories, Intarvin Company, Inc., College Point, New York.

showed a slight decrease in ammonia excretion on the first experimental day followed by an increase on the last experimental day this final amount being larger than that excreted on the last day of the preliminary period. All of the experiments demonstrated that abnormally large quantities of ammonia were eliminated during both the preliminary and experimental days, with the greatest amounts in the latter period. This seems to indicate the presence of excess acids in the body, demanding an increase in ammonia production to neutralize them by the formation of salts. Therefore this high fat diet appears to have caused an excessive formation of acids during the ingestion of intarvin as well as of butter. From these results we may conclude that the intensity of acidosis was increased rather than decreased during the period when intarvin was ingested.

Keefe, Peilzweig and McCann demonstrated a slight decrease in ammonia excretion by diabetic patients when intarvin was added to their diets. The studies of Benedict Ladd, Strauss and West,<sup>1</sup> Lundin<sup>11</sup> and Modern<sup>2</sup> showed a slight increase in ammonia excretion by normal and diabetic subjects on the first day that intarvin was added to a low carbohydrate high fat diet, this increase was followed by a decrease but at no time was the amount of ammonia excreted (during the period of intarvin ingestion) diminished below the smallest amount found during the period of natural fat ingestion.

*Acetone Bodies*—In four of the five experiments (Table I) there was an abrupt rise in the curve of excretion of acetone bodies during the preliminary days, followed by a slight decline on the first day of the experimental periods, this was succeeded by another rise to a point as high as or higher than the level of acetone excretion on the last preliminary day. In Experiment IV instead of the usual slight drop in acetone excretion on the first day of intarvin ingestion, there was a continued increase in the amount of acetone bodies excreted from the beginning to the end of the experiment. The results of this study showed that the substitution of intarvin for butter in the ketogenic diet had no tendency to lessen acetoneuria, but rather intensified this condition.

Contrary to these results Kahn<sup>12</sup>, Benedict Ladd, Strauss and West,<sup>1</sup> Hubbard and Wright,<sup>14</sup> and Keefe, Peilzweig and McCann<sup>1</sup> demonstrated a reduction in acetoneuria when intarvin was fed to normal or diabetic individuals in whom acidosis had been produced. Modern<sup>2</sup> and Lundin<sup>11</sup> showed that the curve of the amounts of acetone bodies excreted reached its highest point on the first day that intarvin was taken, but that this was followed by a gradual decline. However Lyon, Robson and White<sup>1</sup> demonstrated an increase in the amount of acetone bodies in the blood and urine of diabetic patients when intarvin was added to their diets. Sevringhaus<sup>1</sup> found no diminution in the ketonuria of normal subjects when 100 grams of glyceryl margarate were fed.

*Organic Acids*—There was a decided increase in the amounts of organic acids eliminated, beginning with the first day of the preliminary periods and extending through the last day of the experimental periods (Table I). Three of the five curves showed a continuous rise during the entire experiment.

(Experiments II, III, and IV, Table I), one of these three showed a gradual rise for the first four days of the test, followed by an exceedingly sharp rise on the last day of the experimental period (Experiment III, Table I). In the other two cases, there was a very slight decrease in organic acid excretion on the first experimental day, followed by a decided rise on the last day of the test (Experiments I and V, Table I). This study demonstrated that organic acid excretion was abnormally great on a low-carbohydrate high-fat diet, whether the principal fat was butter or intarvin, this acid excretion reached its height when intarvin was the chief fat ingested. These facts may be interpreted as meaning that when this odd carbon fat was metabolized, it formed more organic acids than did the natural fat. At least, the data seem to indicate an increase in the intensity of the acid condition of the body.

Keefe, Perlzweig and McCann<sup>9</sup> found on the addition of intarvin to the diet a decided diminution in organic acid excretion in two out of four diabetic cases, only a slight decrease in one case and a decided increase in the fourth. Lundin's<sup>11</sup> tables gave evidence of an increased organic acid elimination when the subject resumed his diet containing natural fat, after intarvin ingestion. Modern<sup>12</sup> was of the opinion that intarvin is like natural fat in requiring carbohydrates for its complete utilization, and therefore, that it is of no value in practical diabetic treatment. Its incomplete combustion in Modern's experiments resulted in acidosis due to organic acids (lactic and pyruvic) rather than to acetone bodies.

*Titratable Acidity*—The excretion of titratable acids increased during our tests, the curve reaching its highest point either on the last day of butter ingestion or on the first day of intarvin (Table I). This was followed by a slight decrease in acid excretion. Our results are similar to those of Keefe, Perlzweig and McCann<sup>9</sup> and of Lundin,<sup>11</sup> who experimented with diabetic and normal subjects. It is seen in our experiments that large amounts of titratable acids were eliminated during the periods of both intarvin and butter ingestion, there was a tendency toward a slight decline in the curve of acid excretion at the end of the intarvin period. The increases in ammonia, organic acids and acetone bodies indicated that a larger rather than a smaller amount of acid substances was being formed throughout this intarvin period. These acid substances may have been of such a nature that some of them were untitratable within the range of the indicator used for determination of acidity, the great increase in ammonia excretion during intarvin ingestion indicated that a large part of the acid substances was neutralized by ammonia and excreted as ammonium salts, rather than as free acids, in addition toward the end of the period of intarvin ingestion, the acetone bodies may have been excreted chiefly in the form of acetone rather than as acetoacetic and beta-hydroxybutyric acids.

#### ABSORPTION OF THE FATS

The results obtained in Experiment I from the estimation of fat in the feces indicated that approximately 99 per cent of the total fat ingested was absorbed when butter was the principal fat, as compared with 88.5 per cent

TABLE II  
ABSORPTION OF FAT

SUBJECT 1		EXPERIMENT 1				
DATE 1926	DIET	WT OF MOIST STOOLS GM	FAT IN FECES GM	FAT IN FECES PER CENT	PER CENT OF FAT UNABSORBED 1 PER CENT	PER CENT OF FAT ABSORBED PER CENT
May 25	Basal diet and butter	5	17.1	3.21		
May 26	Basal diet and butter	118	2.55	2.16	1	99
May 27	Basal diet and butter	104	0.57	0.34		
May 28	Basal diet and intarvin	327	10.69	3.27		
May 29	Basal diet and intarvin	194	23.6	12.18	11.5	88.5
May 30	Basal diet and intarvin	93	1.33	1.13		

absorbed when the odd carbon atom fat, intarvin was taken (Table II). These fat determinations were not extremely accurate, since it was often difficult to get a representative sample for analysis this was especially true in the period of intarvin ingestion, since some of the fat appeared in the feces in small flakes or lumps. However, these figures give an indication of the relative digestibility of the two fats. From the observations in one experiment, it may be concluded that intarvin was not absorbed quite as completely as was butter. Consequently the calorie value of the diet during the intarvin period may not have been quite high enough to cover the maintenance requirement of the individuals as a result the body fat may have been called upon to make up the slight deficiency. Nevertheless in Experiments IV and V where a larger proportion of the total fat ingested was in the form of the fats under investigation and where a sharper contrast consequently could be made between the effects of these two fats the results were entirely similar to those obtained in the first three experiments.

Our results on the absorption of intarvin agree practically with those of Kahn<sup>1</sup> and Benedict Ladd Strauss and West<sup>10</sup> in the experiments of Keefer, Perlzweig and McCann<sup>9</sup> and Lundin<sup>11</sup> only 80 to 85 per cent of the intarvin ingested was absorbed.

#### SUBJECTIVE EFFECTS

Besides the influence on acid metabolism in the body these diets were not without other effects. During the preliminary days when butter was the principal fat, the subjects noted retardation in digestion resulting in feelings of discomfort, flatulence and nausea. There were symptoms of fatigue, drowsiness, and frequent dull headaches. During the days of intarvin ingestion, these effects were intensified and the subjects found difficulty in performing their laboratory and departmental work. The synthesized fat seemed to have a laxative effect producing gastrointestinal distress, together with loose bowel movements and loss of some undigested fat. However the subjects lost no more in body weight during the intarvin than during the butter period, each individual lost about one pound on each day of the experiment.

#### SUMMARY

Three healthy young women served as subjects in five experiments. They were given diets poor in carbohydrates and rich in fat, most of the fat being in the form of butter during the two or three day preliminary periods and

in the form of glyceryl trimargarate (intarvin) in the three-day experimental periods. Comparison of these two fats was made as to their effects on acidosis produced by the ketogenic diets. Quantitative determinations of creatinin, ammonia, total acidity, organic acids, and acetone bodies were made in twenty-four-hour urine specimens, the percentage of fat absorbed was estimated in one instance.

Large amounts of ammonia, acetone bodies, organic acids and titratable acids were excreted by the subjects in all of the experiments, these quantities were generally larger during the periods of intarvin ingestion. Therefore, intarvin did not appear to act as an antiketogenic substance nor to have the power to lessen the degree of acidosis produced in normal individuals by a high-fat diet, on the contrary, it was probably oxidized in the body with the resulting formation of acid substances which tended to increase the acidosis. Intarvin was slightly less completely absorbed by the intestine than was butter.

## REFERENCES

- <sup>1</sup>Kahn, M. Am Jour Med Sc, 1923, clxvi, 826
- <sup>2</sup>Kahn, M., and Nolan. Medical Staff Meeting, Beth Israel Hospital, New York, August 3, 1923
- <sup>3</sup>Fohn, O. Method Given in Hawk, P. B., and Bergeim, O. Practical Physiological Chemistry, 1926, p. 732
- <sup>4</sup>Van Slyke, D. D., and Palmer, W. W. Jour Biol Chem, 1920, lvi, 567
- <sup>5</sup>Fohn, O., and Bell, R. D. Jour Biol Chem, 1917, xxx, 329
- <sup>6</sup>Fohn, O. Method Given in Hawk, P. B., and Bergeim, O. Practical Physiological Chemistry, 1926, p. 705
- <sup>7</sup>Van Slyke, D. D. Jour Biol Chem, 1917, xxx, 455
- <sup>8</sup>Saxon, G. J. Jour Biol Chem, 1914, xvi, 99
- <sup>9</sup>Keefe, C. S., Perlzweig, W. A., and McCann, W. S. Bull Johns Hopkins Hosp, 1924, xxxi, 265
- <sup>10</sup>Benedict, E. M., Ladd, W. S., Strauss, M. L., and West, R. Proc Soc Exper Biol Med, 1924, xvi, 485
- <sup>11</sup>Lundin, H. Jour Metab Research, 1923, iv, 151
- <sup>12</sup>Modern, F. S. J Metab Research, 1923, iv, 177
- <sup>13</sup>Kahn, M. Arch Int Med, 1925, xlv, 46
- <sup>14</sup>Hubbard, R. S., and Wright, F. R. Proc Soc Exper Biol Med, 1924, xxii, 70
- <sup>15</sup>Lyon, D. M., Robson, W., and White, A. C. Brit Med Jour, 1925, i, 207
- <sup>16</sup>Serringhaus, E. L. Jour Biol Chem, 1924, lvi, Proc lvi

# AN IMMEDIATE FALL OF BLOOD CHOLESTEROL AFTER EATING OR AFTER HISTAMINE INJECTION A STUDY OF ITS PHYSIOLOGIC SIGNIFICANCE\*

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## INTRODUCTION

WHILE working under Dr. Warfield L. Longcope, as a Duke University Medical Research Fellow, at the Johns Hopkins School of Medicine in 1926, I observed a fall in the blood serum cholesterol of a dog immediately after eating a meal. Several repetitions using a modification of Sackett's technique suggested personally by Peisweig, confirmed this phenomenon. Dr. Longcope considered the matter worthy of further attention, but only within the last eight months have I been free to return to it.

The accurate techniques both for blood and tissue cholesterol which I developed for my own use have been described elsewhere,<sup>1</sup> and it need only be stated here that they were well suited to the comparative nature of this investigation, and are dependable to within 2 per cent.

### THE FACT OF AN IMMEDIATE POSTPRANDIAL FALL OF BLOOD CHOLESTEROL

**Control**—It was first necessary to know if eating and if the withdrawal of small blood samples caused any significant changes in blood volume. Since blood volume methods employing a dye stuff are unsuitable where repeated samples are to be taken, *total solids* and *serum proteins* were taken as sufficiently accurate indications. For total solids, 2 c.c. of oxalated whole blood were placed in a weighing bottle weighed at once, dried to a constant weight at 100° C. (usually twenty-four hours), and the bottle again weighed. The serum proteins were estimated by refractometer readings taken at 17.5° C. and worked out from Reiss' table.

**Technic of control experiments**—The following outline shows the method of feeding and blood taking and was essentially the same in all cases, although the times of blood taking might vary by a few minutes in different experiments.

The dog was given no food or liquid on the morning of the test.

- 9:00 A.M. 4 to 6 c.c. blood taken from vein
- 12:00 NOON 4 to 6 c.c. blood taken from vein
- 12:05 P.M. Dog was given one half pound lean beef but no liquid
- 12:20 P.M. 4 to 6 c.c. blood taken from vein
- 1:10 P.M. 4 to 6 c.c. blood taken from vein

The results are shown in Table I.

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TABLE I  
EFFECT OF EATING AND BLOOD TAKING ON BLOOD VOLUME

	9 00 A M SAMPLE	12 00 NOON SAMPLE	12 20 P M SAMPLE	1 50 P M SAMPLE
<i>Experiment I</i>				
Percentage by weight of solids	22.98	23.18	23.30	22.17
Percentage of serum albumin	8.13	8.49	8.10	8.41
<i>Experiment II</i>				
Percentage by weight of solids	22.46	22.35	22.25	22.27
Percentage of serum albumin	7.48	7.16	7.05	7.67
<i>Experiment III</i>				
Percentage by weight of solids	22.65	21.85	21.84	21.78
Percentage of serum albumin	7.40	7.38	7.27	7.35
<i>Experiment IV</i>				
Percentage by weight of solids	21.65	21.54	20.98	21.25
Percentage of serum albumin	7.29	7.07	7.05	7.05

The results indicate that any blood volume changes caused by eating or taking samples of blood are so small as to be insignificant in their bearing on estimations of cholesterol.

The cholesterol determinations were made usually from duplicate analyses of two equal samples of blood and no results recorded where the experimental error exceeded 2 per cent. Only 3 samples were taken, the first before eating, the second soon after eating, and the third at a longer interval. In the following typical protocols, the figures indicate total cholesterol of the whole blood, unless otherwise stated.

*Experiment I*—Dog No. 1, male, Airedale, weight 26 kilo

Sample 1 12 40 P M, 166 mg per 100 cc

Dog fed at 12 48 P M

Sample 2 1 00 P M, 137 mg per 100 cc

Sample 3 3 05 P M, 156 mg per 100 cc

Comment A fall of 29 mg per 100 cc, twelve minutes after meal

*Experiment V*—Dog No. 2, female, mongrel, weight 11 kilo

Sample 1 10 00 A M, 219 mg per 100 cc

Dog fed at 10 03 A M

Sample 2 10 15 A M, 195 mg per 100 cc

Sample 3 10 32 A M, 196 mg per 100 cc

Comment A fall of 24 mg per 100 cc after thirteen minutes and persisting after twenty-nine minutes

*Experiment VI*—Dog No. 3, female, mongrel, weight 12.5 kilo

Sample 1 9 33 A M, 161 mg per 100 cc

Dog fed at 9 38 A M

Sample 2 9 46 A M, 142 mg per 100 cc

Sample 3 9 56 A M, 133 mg per 100 cc

Comment A fall of 28 mg per 100 cc within eighteen minutes after eating

*Experiment VII*—Dog No. 3

Sample 1 9 34 A M, 171 mg per 100 cc

Dog fed at 9 38 A M

Sample 2 9 46 A M, 151 mg per 100 cc

Sample 3 9 52 A M, 139 mg per 100 cc

Comment A total fall of 32 mg per 100 cc over a period of eighteen minutes after eating

*Experiment I*—Dog No 2 (Note Figures represent total plasma cholesterol)  
 Sample 1 12 57 P.M. 165 mg per 100 cc plasma  
 Dog fed at 1 00 P.M.  
 Sample 2 1 11 P.M. 122 mg per 100 cc plasma  
 Sample 3 1 17 P.M. 135 mg per 100 cc plasma  
 Comment A fall of 21 mg per 100 cc plasma during seventeen minutes

It is of interest to note that the injection of histamine either alone or in combination with a meal produced similar results as shown in the following protocols

*Experiment II*—Dog No 1 male mongrel weight 11 kilo  
 Sample 1 1 07 P.M. 146 mg per 100 cc  
 Histamine (0.1 mg to the kilo) subcutaneous  
 control at 1 10 P.M. No food  
 Sample 2 1 15 P.M. 144 mg per 100 cc  
 Sample 3 1 30 P.M. 113 mg per 100 cc

Comment The injection of histamine was followed by a fall of 31 mg per 100 cc, within twenty minutes

*Experiment III*—Dog No 3  
 Sample 1 9 11 A.M. 133 mg per 100 cc blood  
 Histamine (0.2 mg to the kilo at 9 18 A.M.)  
 subcutaneous  
 Dog fed usual one half pound meat at 9 30 A.M.  
 Sample 2 10 10 A.M. 133 mg per 100 cc blood  
 Sample 3 10 20 A.M. 140 mg per 100 cc blood

Comment A fall of 13 mg per 100 cc in twenty one minutes after a meal and histamine

*Experiment IV*—Dog No 7 (Note Dog was starved two days before experiment)  
 Sample 1 10 11 A.M. 213 mg per 100 cc blood  
 Histamine (0.125 mg to the kilo) subcutaneous  
 at 10 18 A.M.  
 Usual meal of meat at 10 20 A.M.  
 Sample 2 10 30 A.M. 198 mg per 100 cc blood  
 Sample 3 10 44 A.M. 191 mg per 100 cc blood  
 Comment A fall of 14 mg per 100 cc blood after twenty seven minutes

From these experiments as well as nearly a dozen other similar ones, the conclusion was drawn that following a meal or histamine injection or both there occurs, during the immediate postprandial period a fall in the total cholesterol of the blood of the order of 20 to 30 mg per 100 cc. There seems to be no reference to this phenomenon in the extensive literature of cholesterol although it is well known that a rise occurs later due to the absorption of cholesterol from the food.

While its significance is not perfectly clear to me I at first suspected that the cholesterol leaving the blood went to the gastric mucosa to prepare that tissue, in some way, for the work of secreting gastric juice.

To determine this point a type of experiment was devised by which the stomach wall could be sampled before and after the injection of histamine and comparative estimations of cholesterol made.

THE TOTAL CHOLESTEROL CONTENT OF THE GASTRIC MUCOSA BEFORE AND AFTER THE  
INJECTION OF HISTAMINE

The technique was to put a fasting dog under ether after taking a preliminary sample of venous blood (Blood Sample 1). The abdomen was then quickly opened, the stomach mobilized into the wound, and its contents washed out through a small stab wound near the greater curvature. By means of two pairs of large broad-ligament clamps, so placed that their points overlapped, a pouch of the anterior wall of the stomach was completely shut off from the circulation and left thus until near the end of the experiment. This pouch constituted Tissue Sample A. The dog was then given histamine subcutaneously. About ten minutes later a second sample of venous blood was taken (Blood Sample 2). After about fifteen minutes more, a final blood sample was taken (Blood Sample 3). Then the gastric juice was drained from the stab wound into a beaker. Next, the second sample of tissue (Tissue Sample B), slightly larger than A, was quickly removed from the posterior curvature. Finally Tissue Sample A, distal to the clamps, was removed and both samples placed in glass-stoppered bottles to prevent loss of weight by evaporation.

*Controls*—Merely putting a dog under ether was shown in 3 experiments not to affect the blood cholesterol level appreciably. The above stomach operation, but with the omission of histamine injection, was carried out, with results as shown in Table II. Any error in tissue cholesterol estimation due to blood contained in the tissue was circumvented by estimating this blood factor, as described in the paper on methods<sup>1</sup> and deducting it from the total. Preparatory to tissue analysis, the mucosa of Tissue Samples A and B was carefully dissected from the muscular coat and at once weighed in the fresh condition, the figures representing wet weight. For results of these experiments see Table II.

From a study of Table II it is seen that where histamine was injected, as in the first 4 cases, the blood cholesterol fell as was to be expected from my earlier experiments, but that in the control experiment where the operation was carried out without histamine the blood cholesterol remained constant. It will be seen, also, that in the histamine experiments there was a slight decrease of tissue cholesterol in Sample B which remained in the circulation. This decrease was of the following order, respectively, 0.009, 0.036, 0.039, and 0.015 per cent, but that in the control experiment, where no histamine was given, the cholesterol figures were only 0.005 per cent apart and in the opposite direction. Finally the greatest difference in the tissue cholesterol figures was in the second and third cases, viz., those in which some appreciable gastric secretion occurred.

It was suggested that since the secreting portion of the mucosa actually lost cholesterol, this substance might be escaping with the gastric juice, just as it does in bile. An experiment planned to settle this point showed only the merest trace of cholesterol (estimated at  $\frac{1}{10}$  mg per 100 cc) in the gastric juice, but this experiment should be repeated in a Pawlow dog.

TABLE II  
THE CHOLESTEROL OF GASTRIC MUCOSA BEFORE AND AFTER HISTAMINE

WT OF DOG (KILO)	HISTAMINE SUBCUTANEOUSLY (GM)	TOTAL CHOLESTEROL MG PER 100 CC BLOOD IN BLOOD SAMPLES			TOTAL CHOLESTEROL IN MUCOSA SAMPLES CORRECTED FOR CONTENT IN BLOOD AND EXPRESSED IN PERCENTAGE BY WEIGHT		GASTRIC SECRETION
		1	2	3	SAMPLE A	SAMPLE B	
6.0	0.06	161	142	146	0.30	0.20	None
17.4	0.03	187	1	166	0.20	0.14	Free HCl = $7 \text{ cc } \frac{N}{20} \text{ NaOH}$ Combined acid = $\frac{N}{20} \text{ NaOH}$ 42 cc $\frac{N}{20} \text{ NaOH}$
12.7	0.04	15	—	170	0.10	0.10	Free HCl = $1 \text{ cc } \frac{N}{20} \text{ NaOH}$ Combined = $66 \text{ cc } \frac{N}{20} \text{ NaOH}$
19.0	0.05	180	100	111	0.28	0.13	None
Control dog no histamine		182	186	184	0.14	0.30	None

Another suggestion was that although the blood cholesterol shows a fall, this depression is the resultant of two opposite influences and that certain tissues might actually be releasing cholesterol to the blood stream in view of a marked deposition elsewhere.

Since it is well known that cholesterol is excreted in the bile, it was considered wise to study the cholesterol content of the liver after histamine injection. An experiment was devised whereby the liver could be sampled before and after histamine injection. This was accomplished by the use of elastic bands clamped tightly about the lobules which project from the anterior edge of the organ and cutting off the sample distal to this point of constriction. The intervals of blood taking and tissue sampling were similar to those in the stomach experiments, the histamine being given after the first samples of each had been taken, and the other samples removed at fifteen to twenty minute intervals after the injection in such a way that blood samples were nearly simultaneous with tissue samples of the same number, e.g., Blood Sample 2 was taken at the same time as Tissue Sample B, etc. (For results see Table III.)

Obviously these results show no detectable uniformity, such as one would require to envisage a physiologic process, in some an increase, in some a decrease, and in some a stationary level was obtained. It seemed still quite possible however, that the liver might be the organ chiefly responsible for removing the cholesterol from the blood after an injection of histamine. Possibly cholesterol shares with glycogen a nonuniform distribution within this organ. Again, as Grigaut has long believed, cholesterol may be rapidly changed in the liver perhaps oxidized to cholic acid. Either or both of these two possibilities would, of course, render any intelligent comparative estimations extremely difficult of accomplishment.

TABLE III  
THE CHOLESTEROL OF THE LIVER BEFORE AND AFTER HISTAMINE INJECTION

WT OF DOG (KILO)	HISTAMINE SUBCUTANEOUSLY (CM)	TOTAL CHOLESTEROL, MG PER 100 CC BLOOD IN BLOOD SAMPLE			TOTAL CHOLESTEROL IN MUCOSA, SAMPLES CORRECTED FOR CONTAINED BLOOD AND EXPRESSED IN PERCENTAGE BY WEIGHT			GASTRIC SECRETION
		1	2	3	A	B	C	
15.5	0.03	161	148	148	0.266	0.285	0.253	Free HCl = 65 cc $\frac{N}{20}$ -NaOH Combined = 25 cc $\frac{N}{20}$ -NaOH
13.2	0.03	195	137	140	0.221	0.212	0.220	Free HCl = 30.0 cc $\frac{N}{20}$ -NaOH Combined = 12.7 cc $\frac{N}{20}$ -NaOH
16.0	0.04	223	168	153	0.215	0.286	0.260	Free HCl = 55.0 cc $\frac{N}{20}$ -NaOH Combined = 12.4 cc $\frac{N}{20}$ -NaOH
16.0	0.03	140	115	116	0.226	1.187	0.213	Not examined

I then decided to find out what would happen if histamine were given to an animal in whom the liver circulation was largely tied off or diverted.

*Technic*—From a dog under ether a preliminary sample of blood (Blood Sample 1) was taken. The abdomen was opened and, as rapidly as possible, a curved, brass, paraffin-coated cannula was inserted between the portal vein and the inferior vena cava. The portal vein was then tied between this point and the liver and the hepatic artery found and ligated. The congestion of the visceral area caused by the initial clamping, while doing the cannulization, rapidly improved once the cannula was functioning, although some cyanosis remained throughout the experiments. Histamine was not given until the congestion of the viscera had visibly improved, usually at a period of forty minutes from the time of taking the first blood. Thereafter Samples 2 and 3 were taken at fifteen to twenty minute intervals. Total solids were done on the blood samples and although only slight variations were noted these have been taken into consideration in tabulating the cholesterol figures for the blood. (For results see Table IV.)

These figures indicate a virtually level condition of the blood cholesterol. The slight fall in two of the cases is in no way comparable to the greater fall

TABLE IV

THE BEHAVIOR OF BLOOD CHOLESTEROL AFTER HISTAMINE INJECTION WHEN THE LIVER IS LARGELY EXCLUDED FROM CIRCULATION

WEIGHT OF DOG (KILO)	HISTAMINE INJECTIONS (GM)	TOTAL CHOLESTEROL (MG PER 100 CC BLOOD) IN BLOOD SAMPLES		
		1	2	3
10.5	0.05	176	171	176
11.7	0.04	181	180	188
14.5	0.03	169	164	(Spoiled)
20.0	0.03	180	---	175
10.0	0.03	193	203	190

seen in instances where the liver is intact. Possibly in an hepatectomized dog even this slight fall would not occur, since as long as the liver remains in the body some slight circulation takes place in it chiefly from the back flow from the inferior vena cava. These experiments with portal vein cannulae to the inferior vena cava are not considered crucial experiments to settle the question which has been advanced by this paper but they suggest in a broad way that the liver is the organ which either directly or indirectly, removes cholesterol from the blood after an injection of histamine.

Adopting this as a working hypothesis the question arises, why does cholesterol go to the liver? One cannot avoid the idea that this determination of cholesterol to the liver subserves in some way the general process of alimentation. It is associated with eating and with the injection of a well known gastric excitant histamine. That it is more or less specific to alimentation is suggested by a further series of experiments in which one can show that no such fate of blood cholesterol accompanies other well known types of liver activity, for example glycogenesis and glycogenolysis.

#### EXPERIMENT TO DETERMINE IF DURING GLYCOGENESIS, ANY CHANGE OCCURS IN THE LEVEL OF THE BLOOD CHOLESTEROL

*Technic*—A fasting dog was given an intravenous injection of 90 cc of 20 per cent glucose blood samples being taken before and at twenty three and fifty seven minutes after the injection. These three blood samples were subjected to glucose and cholesterol estimations.

	Glucose	Total Cholesterol
Sample 1	0.031 per cent	132 mg per 100 cc
Sample 2	0.248 per cent	178 mg per 100 cc
Sample 3	0.089 per cent	133 mg per 100 cc

The percentages of total solids in the three specimens ran 25.2, 25.8 and 25.8, and the variations in the cholesterol figures lie within the experimental error.

#### EXPERIMENT TO DETERMINE IF DURING GLYCOGENOLYSIS ANY CHANGE OCCURS IN THE LEVEL OF THE BLOOD CHOLESTEROL

*Technic*—A dog was fed large quantities of glucose for two days previous to the experiment. An initial blood sample was then taken on the fasting dog. Then 0.36 mg of adrenalin was injected subcutaneously. After three and one half minutes a second sample of blood was taken. Two minutes later a second equal dose of adrenalin was given. Two further blood samples were taken at five and fifteen minute intervals.

	Glucose	Total Cholesterol
Sample 1	0.081 per cent	142 mg per 100 cc
Sample 2	0.087 per cent	
Sample 3	0.082 per cent	140 mg per 100 cc
Sample 4	0.081 per cent	148 mg per 100 cc

The percentage of total solids in the first and last two specimens ran 26.1, 23.2, and 25.1.

These two experiments indicate that in its activities relative to glucose the liver may function without attracting cholesterol from the blood

Even a meal of 190 grams of pure glucose and nothing else caused in a dog the following cholesterol blood changes

Sample 1	142 mg per 100 cc	(before eating)
Sample 2	147 mg per 100 cc	(after fourteen minutes)
Sample 3	138 mg per 100 cc	(after twenty five minutes)

which indicate that it is the act of eating, rather than any particular character of the food which occasions the fall in blood cholesterol

Is the determination of cholesterol to the liver preparatory to the secretion of bile? I have injected histamine and noted the bile flow from a gall bladder fistula, but although the blood cholesterol underwent a characteristic fall there was no increased bile flow and the amount of cholesterol in the bile did not increase and was of very negligible amount. Bile salts are about our best so-called cholagogue, but the injection of 10 cc of a 10 per cent solution of sodium cholate caused, in my hands, an *increase* of blood cholesterol from 143 to 161 mg per 100 cc of blood. This is in accordance with our conception of cholesterol as a protective antihemolytic substance, being evoked in response to the administration of a hemolytic salt

#### DISCUSSION

The only practical point arising from this work is the fact that eating or injecting histamine causes a fall in blood cholesterol of from 10 to 30 per cent. This point should be taken into consideration in all estimations on animals or on man. (Incidentally, human blood shows an identical behavior after the subject has eaten, as I may point out in a subsequent paper.) So far as can be judged at present, there is considerable evidence that most, or perhaps all of the cholesterol leaving the blood goes to the liver. The reason for this hepatic determination of cholesterol is obscure but it seems to be associated with the process of alimentation and may occur independently of any gastric secretion.

#### CONCLUSIONS

1 The total cholesterol of the blood of the dog falls from 10 to 30 per cent within ten to thirty minutes after eating or after the injection of histamine

2 Some evidence is presented to suggest that the cholesterol which leaves the blood goes to the liver

3 The significance of this hepatic determination of cholesterol is not clear, but it seems associated specially with the bodily function of alimentation

#### REFERENCES

- <sup>1</sup>Cornell, B. S. *Accurate Colorimetric Technique for Blood and Tissue Cholesterol Estimations*, JOUR. LAB. AND CLIN. MED., December, 1928, p. 251
- <sup>2</sup>Reiss, E. Chapter on "Refraktometrische in der Biologie," Abderhalden's *Biochem. Arbeitsmethoden*, VIII, pp. 84 et seq.

# BILIRUBIN A NONTOXIC SUBSTANCE

## EXPERIMENTAL STUDY OF BILIRUBIN IN HEART LUNG PREPARATIONS

By O H HORRALL M D PH D CHICAGO

**B**ILIRUBIN has been referred to frequently as the toxic substance in bile (Bouchard<sup>1</sup> King and Stewart<sup>2</sup> King, Biglow and Pierce<sup>3</sup>) To it has been attributed the deleterious symptoms found in the various types of jaundice such as itching, bradycardia irregular heart, and albuminuria

Two patients with jaundice came to my office recently One had slightly pigmented sclerae and skin There was a small amount of pigment in the urine and blood serum but she had intense itching, definite bradycardia, and marked irregularity of the heart The other patient had a very intense jaundice, the sclerae, skin, urine, and blood serum were very highly colored She had no itching, the heart was regular the rate was normal and the urine contained no albumin These two patients were normal when examined a few months previously and there was no other intervening illness There was a marked inverse proportion between the physical findings and the intensity of the pigmentation

Previous studies<sup>4</sup> have been made with bile and the various constituents of bile, but it was thought advisable to eliminate as many variables as possible and to test the action of pure bilirubin on the heart in heart lung preparations This preparation prevents the action of the liver kidneys, or other organs either as a modifying, detoxicating, or extracting agent of bile pigments when circulating in the blood

The bilirubin was prepared from beef gallstones by a modification of the method of Orndorff and Feeple<sup>5</sup> The purity of the bilirubin was determined by the spectrophotometric chemical, and microscopic methods The bilirubin with which this work was done was of a very high degree of purity and is the same chemical substance as that found in the blood serums of humans and dogs with jaundice and also that found in normal blood

The apparatus used in these experiments was essentially the same as that used by Knowlton and Starling and Lambert and Rosenthal<sup>7</sup> It was modified and adapted for use with one dog The apparatus consisted of a venous reservoir with a tube leading from the brachiocephalic artery to it and another tube leading from the reservoir to the superior vena cava A three way cannula was inserted and securely tied in the brachiocephalic artery or aorta One way went to a mercury manometer which recorded the heart action and blood pressure on a kymograph The tube leading to the

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reservoir passed through an air-tight pressure chamber in which mercury measured the rubber tubing. This glass pressure chamber had two side tubes, one connected with a second mercury manometer, and the other with a rubber bulb for changing the pressure. When the pressure was set at the place desired, the tube leading to the bulb was clamped, thus a constant arterial resistance was established and maintained. Between the resistance chamber and the venous reservoir was placed an inverted 250 cc flask which acted as an air cushion causing a more even flow of blood into the reservoir. The reservoir was of glass and had a capacity of 500 cc. There was a large opening at the upper end and a smaller one at the lower from which a tube passed through the lower end of the water chamber. The temperature of the water in the water-jacket was maintained by tap water flow and also by two electric light bulbs which were regulated by a thermostat also immersed in the same water. The blood passed from the venous reservoir by way of glass tubing and rubber tubing to the superior vena cava through a second three-way cannula. The third way contained a thermometer which recorded the temperature of the blood just as it entered the heart.

The dog was anesthetized with ether,\* the vagi isolated, trachea cannulated, artificial respiration begun, and the vagi sectioned. The respiration was maintained at a constant volume and uniform rate by an automatic machine connected with the air pressure system. The chest was opened on the left side and the clavicle and all the ribs were cut down to the diaphragm so that there was good exposure of the mediastinum. The internal mamillary vessels were tied and cut, the phrenic nerves were sectioned, left subclavian artery and azygos vein were ligated. Ligatures were placed around the superior vena cava, brachiocephalic artery, inferior vena cava and aorta.

Then twenty-five milligrams of heparin (Howell<sup>8</sup>) per kilogram of weight of the dog were dissolved in ten cubic centimeters of physiologic salt solution and injected intravenously. This amount of heparin was found sufficient to prevent clotting during the entire experiment. It is extremely important to see that all bleeding has been stopped. The extracorporeal system was tested out to see that the tubing and vessels were of the proper temperature and could be maintained. The heparin having thoroughly circulated, the salt solution was emptied from the reservoir system and tubing. The brachiocephalic artery was ligated and the arterial cannula inserted and securely tied distal to the clamped vessel. This clamp was immediately removed and the heart allowed to pump about one hundred fifty cubic centimeters of blood into the reservoir, then a clamp was placed on the tubing distal to the cannula. The return tubing and cannula were allowed to fill with blood from the reservoir and clamped. It was important that this tubing contained no air. The superior vena cava was ligated and a clamp placed on it near the heart, then the venous cannula was inserted and tied. A screw clamp was put on the rubber tubing and

\*Several methods of anesthesia were attempted, such as intravenous and intraperitoneal sodium barbital, also mixture of two parts of trichloro-isopropyl-alcohol (isopral) and one part of methyl-propyl-carbinol-urethan (hedonal) intraperitoneally. The dog's heart in this preparation appeared to react unsatisfactorily to these drugs.

the other clamp removed from the vein. The clamp on the arterial system was again removed and the heart allowed to pump blood continually into the reservoir, then the ligature about the aorta was tied, the abdomen compressed and the inferior vena cava was slowly occluded as the screw clamp on the venous rubber tubing was slowly opened thus completing the transition to the heart lung preparation. This change must be made quickly and accurately. Extreme care must be used to tie off all small vessels which may cause loss of blood from the new system. Frequently small anomalous vessels were found on the posterior surface of the proximal portions of the superior vena cava and the brachiocephalic artery, also on the other ligated vessels.

The rate of blood flow must be carefully regulated so that the blood level in the reservoir is constant. When this is constant it is apparent that the heart is receiving and discharging blood satisfactorily, neither being overloaded nor underworked. This flow can be established by carefully balancing the pressure system on the arterial side which regulates the efferent flow of blood, with the size of the lumen of the tubing on the afferent side. This latter is regulated with a screw clamp. The chest is now closed and electric pads applied to the body of the dog to aid in maintaining the temperature of the heart and lungs.

By this method the complete system can be maintained with two hundred to three hundred cubic centimeters of blood—that is the blood from the one dog. The preparation can be kept working much for two to four hours. The blood was autogenous and contains no extraneous substances except whatever ether may remain from the anesthetization and the heparin which was dissolved in ten cubic centimeters of salt solution.

The bilirubin was dissolved in various solutions such as sodium carbonate one per cent, sodium hydroxide one per cent human serum and dog serum. The solutions were warmed to the temperature of the blood and poured into the reservoir or injected into the rubber tubing on the venous side by means of a syringe and needle.

Bilirubin was put into the blood in nine dogs with heart lung preparations. The concentrations varied but reached a maximum of 22 grams per liter. A larger amount could not be used because the quantity of sodium carbonate necessary to dissolve it would become so great that the increase in the blood alkalinity would itself affect the heart. The quantity of sodium carbonate added to the blood in these experiments when tested by itself in the control experiments did not affect the heart. Observations with the van den Berg reaction have showed that bilirubin is present in normal human blood serum in amount of 1 to 2 mg for each 100 cc according to Greene Snell, Walters and Roundtree<sup>9</sup>. They also state that in obstructive jaundice the serum bilirubin with direct test may rise as high as 30 mg for each 100 cc. Greene and Conner<sup>10</sup> report one case following a hemolytic crisis with an increase of serum bilirubin to 26.2 mg for 100 cc blood.

There were slight variations in the pulse rate corresponding to the usual





variations in the control heart-lung preparations. The blood pressure did not change in any instance. The fat about the heart also the lungs became deeply colored, and on letting the blood cells separate from the serum the latter had a deep orange appearance.

For comparison and to see that the heart would react as in the intravenous method, sodium glycocholate 0.662 per cent caused immediate fall in blood pressure, irregular heart cessation of activity, with acute dilatation of the whole heart.

Sodium cholate 0.47 per cent caused irregular heart action and fall in blood pressure.

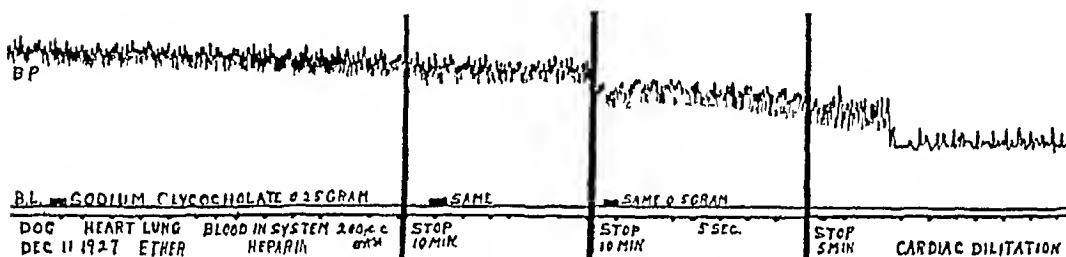


Fig. 1.—Dog heart-lung preparation. Sodium glycocholate 1.25 grams per 1000 cc of blood caused slow fall in blood pressure and rate. Five grams per 1000 cc caused acute cardiac dilatation within six minutes.

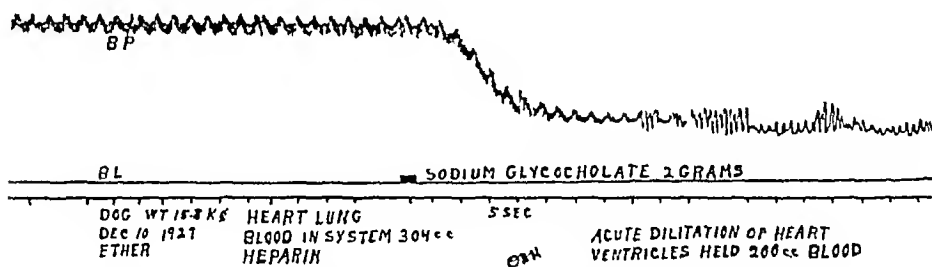


Fig. 2.—Heart-lung preparation. Sodium glycocholate 6.57 grams per 1000 cc blood caused a fall of blood pressure in ten seconds. This was accompanied by irregular heart action and rapid acute dilatation of the heart.

Whole gall bladder bile, 5 cc in 210 cc blood, caused irregular heart with greatly increased amplitude of beat. Atropine then caused slowing of the rate, adrenalin had very slight effect, while a second injection of bile caused a marked increase in amplitude of beat, also increase of the rate, but this was soon followed by cessation of heart action. What appeared at first to be the stimulating action of bile, quickly paralyzed the heart.

The hearts in these heart-lung preparations of dogs did not demonstrate any action of bilirubin, neither as a stimulant nor depressant. The same preparations and similar preparations showed definite effects from whole gall bladder bile, sodium glycocholate and sodium cholate. The toxic portion of

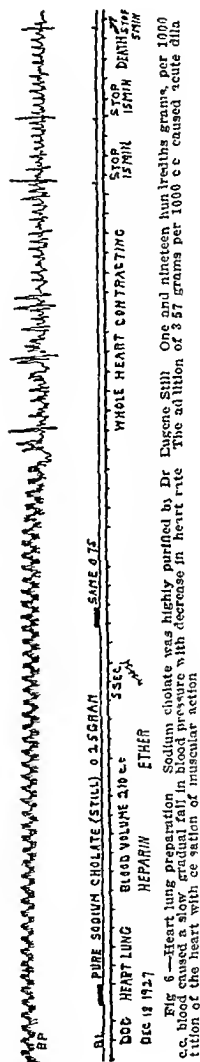


TABLE I

DOG	BLOOD VOLUME		BILIRUBIN PER CENT		HEART RATE MINUTES					BLOOD PRESSURE MINUTES				
	CC	GM	CO	C	BEFORE	2	5	10	30	BEFORE	2	5	10	30
1	250	0.1	0.04		150	150	150	150	150	144	144	144	144	144
2	300	0.2			168	174	180	178		120	120	120	120	
		0.1			180	180	192	180		116	114	112	112	
		0.3	0.20		170	168	176	180	170	118	116	116	116	112
3	210	0.25	0.11		132	132	120	120	126	144	144	144	144	144
4	180	0.25	0.11		180	174	176	180	180	120	120	120	120	120
5	250	0.3	0.5		156	156	156	156	156	100	100	100	100	100
6	200	0.1	0.05		120	120	114	114	114	100	100	100	100	100
7	180	0.1			204	204	210	198		94	94	94	94	
		0.1			224	216	204			94	94	94		
		0.1			190	204	204			94	94	94		
		0.1	0.22		198	204	210	204	198	94	94	94	94	94
8	240	0.2			186	180	180	174		162	162	162	164	
		0.2			174	174	174	168	172	164	164	164	166	166
		0.1	0.207		172	174	174	178	178	170	165	165	165	165
9	150	0.05			144	150	150			106	106	106		
		0.05	0.06		147	147	144	144	144	106	106	106	106	106

the bile (cholate radical) caused a fall in the blood pressure, diminution in the heart rate, increased amplitude of beat followed by decreased amplitude, irregular rhythm, and acute dilatation of the auricles and ventricles

## CONCLUSION

Bilirubin has no effect on the heart

## REFERENCES

- \*King and Stewart Jour Exper Med, 1909, vi, 673  
 \*Bouchard (a) See Stedeman, Ztschr f Biol, 1896, xxxiv, 57 (b) Leçons sur les Auto-intoxications des les Maladies, 1887  
 \*King, Biglow, and Pierce Jour Exper Med, 1912, xv, 159  
 \*Horrall, O H, and Carlson, A J The Toxic Factor in Bile, Am Jour Physiol, July, 1928, lxxv, No 3, p 591  
 \*Orndorff, and Teeple Am Chem Jour, 1905, xxxvii, 215  
 \*Knowlton and Stirling Jour Physiol, 1912, xiv, 206  
 \*Lambert, R K, and Rosenthal, A H Am Jour Physiol, May, 1927, lxx, No 3, 710  
 \*Howell Am Jour Physiol, 1925, xiv, 553  
 \*Greene, Snell, Walters, and Roundtree Collected Papers of the Mayo Clinic, 1924, vii, 200  
 \*Greene and Commer Arch Int Med, 1926, lxxvi, 167

# HEMOAGGLUTINATION

## II HEMOAGGLUTINATION IN THE BLOOD OF BOVINES\*

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MANY investigators have studied agglutination in the blood of animals and although the clumping phenomenon has been frequently observed, serious effort to establish types has met with small success. The laboratory findings, for the most part, have been contradictory and no satisfactory groupings have been definitely determined.

### HISTORY

Heltoen, 1907, after a study of cross agglutination in a series of cases in animals, including horses, cattle, dogs, guinea pigs and rabbits, was not able to find any isoreagglutination. Ottenberg and Friedman, 1911, found three groups in steers. Ingebrigsten, 1912, found five positive agglutinations in a series of typings of forty cats. He could not, however, establish any fixed groups. Fishbein, 1913, found occasional agglutinations in rabbits, sheep, swine and cattle, but none in frogs. The clumpings appeared far less common in animals than in man and seemed to bear no relation to color or race. Weszchky, 1920, could find no isoreagglutination in rabbits, guinea pigs, chickens, or cattle but did find clumping in the blood of swine, dogs and horses. Landsteiner and van der Scheer, 1924, found three groups in horses. Walsh, 1924, also found isoreagglutination in horses but could establish no definite types. Snyder, 1924, could detect no clumping in rabbits, although he tested most of the common strains. Landsteiner and Miller, 1925, in typing monkeys and anthropoid apes established four groups, almost identical with those found in man. With the exception therefore of this last group of animals, little has been definitely settled and until many more typings have been made and those on a much larger scale the question must remain in its present chaotic state.

### TECHNIC

The technique employed in this paper follows in general, the technique outlined in my former paper, "I. Hemagglutination in the Blood of Infants."

I. The open slide agglutination method was employed. All readings were made by the microscope.

II. The bovine blood was secured from two sources: the thoroughbred Holstein herd of the Western Washington Experiment Station, and the beef cattle of the Frue Packing Co.

III. The blood samples were collected in paired, two dram vials: one empty, the other partly filled with 1 per cent sodium citrate in normal salt

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solution All readings were made while the specimens were fresh, i e, within forty-eight hours after collection

In cross-typing one bovine blood with another the following isoagglutination results were obtained

## ISOAGGLUTINATION

TABLE I

RESULTS OF CROSS TYPING BOVINE RED CELLS WITH BOVINE SERUM, HOLSTEIN AGAINST HOLSTEIN AND DURHAM AGAINST DURHAM

SERUM X O R C X	CASES	SERUM Y O R C Y	PER CENT
Both positive	0		0
One positive	0		0
Neither positive	100		100

Table I shows a series of one hundred cross-typings between animals of a certain similar strain of bovines The number of tests made on Holstein and Durham were practically equal All tests proved negative for agglutination

TABLE II

RESULTS OF CROSS TYPING BOVINE RED CELLS WITH BOVINE SERUM, DURHAM AGAINST HOLSTEIN

SERUM H O R C D	CASES	SERUM D O R C H	PER CENT
Both positive	0		0
One positive	16		20
Neither positive	64		80
Total	80		100

Table II shows a series of eighty cases of cross-typing between Durham and Holstein bloods In no case did both tests prove positive In only sixteen tests did one show positive, and in each positive instance it was the Durham red cells that clumped Here, two animals showed agglutination constantly when their red cells were typed with Holstein sera, they would not, however, agglutinate each other This is shown best, perhaps, in Table III

TABLE III

DETAILED TABULATION OF CROSS TYPING SHOWN IN TABLE II  
HOLSTEIN SERA

		1	2	3	4	5	6	7	8
Durham Red Cells	A	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-
	C	+	+	+	+	+	+	+	+
	D	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-
	F	-	-	-	-	-	-	-	-
	G	-	-	-	-	-	-	-	-
	H	-	-	-	-	-	-	-	-
	I	+	+	+	+	+	+	+	+
	J	-	-	-	-	-	-	-	-

It will be seen that Table III, shown above, agrees, in general, with Table IV of the Fishbein series on "Isoagglutination in Cattle." The red cells C and I of the beef cattle, in the above series, correspond quite closely to the red cells No IX as shown in his series. No lack of agglutination was observed in the above instances neither was occasional sporadic clumping found perhaps a larger series would show their presence.

It appears from the above findings, that there are two or more types of bovine blood. First a large group constituting the major percentage which does not agglutinate. It is not agglutinated by the sera of bloods which its own sera clumps. Second, a small group that does agglutinate when its red cells are mixed with sera from the large group named above, but whose own serum cannot cause agglutination.

It appears certain that the phenomenon of agglutination is far less common in bovine blood than in human blood. The reason is probably largely mathematical, i.e. due to the pronounced unequal grouping that obtains in bovine blood.

It has been shown that when the erythrocytes of one animal are mixed with the blood serum of another but from widely different species, clumping is a fairly constant phenomenon. It is known as heteroagglutination and is treated under Tables IV and V.

## HETEROAGGLUTINATION

TABLE IV

RESULTS OF AGGLUTINATION OF HUMAN RED CELLS WITH BOVINE SERUM

NO. CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
100	100	0	100	0

Of the one hundred cases shown in Table IV above, where human red cells were mixed with bovine serum all tests proved positive. It shows 100 per cent heteroagglutination.

TABLE V

RESULTS OF AGGLUTINATION OF BOVINE RED CELLS WITH TYPES II AND III ADULT HUMAN SERA

TYPES	NO. CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
II	88	85	3	94	6
III	70	66	4	94	6

In the series shown in Table V, bovine red cells were mixed, respectively, with types II and III adult human sera. It will be seen that in both types of sera 6 per cent of the tests proved negative. At least another 6 per cent, for each type of sera used, showed unusually weak agglutination with markedly delayed reactions. Routine intermittent agitation would clump these red cells into the usual characteristic groupings, only to be dispersed again by a gradual diffusion during the quiet interim. The same

phenomenon was observed when strong agglutinating sera were diluted a dozen or more times with isotonic salt solution, then mixed with red cells and agitated as above

### CONCLUSIONS

1 Isoagglutination is found in the blood of bovines, although less common than in the blood of man

2 Isoagglutination seems to occur less frequently between bovine animals of similar strain than it does among those of widely divergent strains

3 The groupings appear to be very unequally proportioned, a single group comprising the vast majority of individuals, thus naturally accounts for the fewer number of agglutinations encountered

4 Heteroagglutination, observed when bovine serum is mixed with human red cells, appears to be a constant phenomenon, it does not always occur when bovine red cells are mixed with human serum

### REFERENCES

#### PART I

- De Brasi, Bruno Isoagglutinin in the Blood of the New Born, Jour Am Med Assn Nov 24, 1923, lxxvi, 1776
- Hooker, S B, and Anderson, L M The Specific Antigenic Properties of the Four Groups of Human Erythrocytes, Jour Immunol, 1921, vi, 419
- Kolmer, J A Infection, Immunity and Biologic Therapy, W B Saunders Co, Philadelphia 1924
- Landsteiner, K, and von der Schuer, J Serological Examination of a Species Hybrid (1 On the Inheritance of Species-Specific Qualities), Jour Immunol, 1924, ix, 213
- Sandford A H Isohemagglutination Groups, Jour Am Med Assn, April 27, 1918, lxx, 1221
- Snyder, L H Blood Grouping and Its Practical Applications, Arch Path and Lab Med August, 1927, ix, 215-57
- Vincent, B Agglutination Test Jour Am Med Assn, April 27, 1918, lxx, 1219

#### PART II

- Fishbein, M Isoagglutination in Man and Lower Animals, Jour Infect Dis, 1913, xii, 133
- Hecktoen, L Isoagglutination of Human Corpuseles, Jour Infect Dis, 1907, ix, 297
- Ingebrigt, R Die Bedeutung der Isoagglutinine für die Schicksale Homoplastisch Transplantierten Arterien, München med Wchrschr 1912, lx, 1475
- Landsteiner, K, and von der Schuer, J Serological Examination of a Species Hybrid (2 Tests With Normal Agglutinin), Jour Immunol, 1924, ix, 221
- Landsteiner, K, and Miller, C P Serological Studies on the Blood of Primates (1 The Differentiation of Human and Anthropoid Bloods), Jour Exper Med, 1925, xlii, 841
- Ottenberg, R, and Friedman, S S The Occurrence of Grouped Isoagglutination in the Lower Animals, Jour Exper Med, 1911, xvi, 531
- Snyder, L H Isohemagglutinin in Rabbits, Jour Immunol, 1924, ix, 45
- Walsh, L S N The Blood Interrelationship of Horses, Asses and Mules, Jour Immunol, 1924, ix, 49
- Weszecky, O Untersuchungen über die gruppenweise Hamagglutination beim Menschen, Biochem Ztschr, 1920, cxxii, 159

# LABORATORY METHODS

## OBSERVATIONS ON THE DIAZO TEST IN NEPHRITIS\*

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SINCE the accidental discovery by Andrews<sup>1</sup> that an orange buff color appears when the Van den Bergh test is applied to serum of nephritic individuals, several other investigators made further study of the phenomenon, with the hope of ascertaining the nature of the chemical reaction responsible for this color change and its clinical significance. Several months after Andrews' discovery, Becker<sup>2</sup> working independently found a positive diazo reaction on the serum of patients suffering from severe nephritis or uremia. This observer concluded that certain aromatic acids present in the blood of these patients were responsible for this color reaction. He stated that the acids are formed in the intestines and excreted through the kidneys when the latter function satisfactorily but accumulate in the blood in cases of renal insufficiency.

Hewitt<sup>3</sup> found a positive diazo test in each of a series of twelve cases of uremia. He suggests that the phenomenon may be due to cyclic amines, such as histamine and tyramine. He also is of the opinion that the accumulation of these products in the blood is due to poor elimination by the kidneys. Hewitt shortened and simplified the test so that it can be performed in a few minutes. The technique is as follows:

Two cc. of 90 per cent alcohol are added to one cc. of blood plasma or serum and the mixture is centrifuged or filtered. One cc. of this supernatant fluid or filtrate is removed with a pipette. To this one cc. of clear fluid is added 0.5 cc. of alcohol and 0.25 cc. of freshly prepared diazo reagent. The mixture is boiled for thirty seconds then a few drops of sodium hydroxide (10 per cent) are added. A rapid development of a pink color indicates a positive reaction. This pink color may disappear rapidly.

Rabinowitch,<sup>4</sup> from a study of 8 cases in which the diazo test was found to be positive, concluded that reaction is found only in blood from subjects showing marked retention of waste products, with lesions such as advanced chronic nephritis or acute surgical conditions of the kidneys pyonephritis, etc. In a control series of 12 cases, suffering mainly from arterial diseases,

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The diazo reagent is prepared from two separate solutions A and B each of which keeps well

Sol. A consists of 1 gm. sulphanilic acid, 1 cc. concentrated hydrochloric acid and 100 cc. distilled water

Sol. B consists of 0.5 gm. sodium nitrite in 100 cc. distilled water

Reagent consists of 2 cc. of solution A and 0.5 cc. of solution B freely mixed

the reaction was negative. This investigator called attention to the observation made that a positive reaction is not always associated with a fatal prognosis. In this connection he cited a case of nephritis complicating pregnancy with a positive diazo reaction which, after subsidence of the acute stage, became negative and the patient recovered. Three other cases were described from which the author concluded that the diazo reaction is of a greater prognostic significance as regards the time factor than the creatinine in chronic nephritis.

More recently, Blotner and Fitz applied the diazo test to the blood of several hundred patients with various diseases. They also concluded that in no case without renal insufficiency was even a mildly positive test obtained. The test was found positive, however, in 36 patients with advanced nephritis and renal insufficiency. They concluded from their observations "that a positive diazo reaction is found only in cases of profound renal insufficiency and does not occur in other conditions." The authors also stated that a positive test is a very ominous sign and is usually followed by a fatal outcome within a short space of time. They did add, however, that a positive test may be present for a long interval without the immediate development of a fatal uremia. Another of their observations was that the phenolsulphonephthalein output in positive cases was always low and that this functional test more nearly paralleled the positive diazo than did the blood urea.

From the contents of the foregoing paragraphs, therefore, the following conclusions may be drawn concerning the diazo test on the blood serum or plasma:

- 1 The test is positive only on the blood of those subjects that show marked retention of waste products and not in any other condition. This opinion is emphatically expressed by all the above mentioned investigators.

- 2 A positive test is always associated with poor renal function as determined by the various tests at present available, such as the phenolsulphonephthalein and the urea concentration tests. This is especially pointed out in the papers of Rabinowitch and also of Blotner and Fitz.

- 3 A positive test is an ominous sign and is usually associated with a fatal outcome within a short time after the test is found to be positive.

Being impressed with the need for further study on this subject, it was deemed advisable to secure further data from a greater number of cases than was heretofore studied. Accordingly, observations have been made on more than a thousand cases including a variety of diseases. The method employed was the routine examination for the diazo reaction of all bloods sent to the laboratory from the hospital and dispensary. In this way positive reactions were discovered where the clinical or laboratory evidence did not indicate the possibility of such findings. Separate records were kept of all patients whose bloods showed a definite accumulation of waste products, but whose blood serum or plasma gave a negative diazo reaction. Some of these patients died of clinical uremia as will be seen from Table I.

TABLE I  
POSITIVE DIAZO CASES

CASE NO	MG PER 100 CC UREA N	MO PER 100 CC CREATININ	RENAL FUNCTION TESTS	BLOOD PRESSURE	AGE	CLINICAL DIAGNOSIS
1	60	5.0	1 lithalein trace	160/60	35	Subacute and chronic glomerulo nephritis
2	50	5.0	Phthalein trace	180/90	40	Chronic glomerulo nephritis and uremia
3	27	4.3	Phthalein trace	230/120	49	Chronic glomerulo nephritis
4	70	6.0	Phthalein trace	150/70	60	Chronic glomerulo nephritis and gout
5	78	7.2	Phthalein trace	180/80	25	Chronic nephritis
6	32	4.5	Phthalein trace	190/110	49	Chronic nephritis
7	1-	1.4	Phthalein 21 per cent in two hr	160/80	40	Cerebral thrombosis
8	6-	2.3	Phthalein 50 per cent in two hr	190/70	67	Ureteral stricture pyelitis
9	2-	3.7	Phthalein trace	140/00	10	diabetes
10	78	6.0	1 lithalein trace	210/100	67	Hypertension and chronic nephritis
11	35	4.6	Phthalein 10 per cent in two hr	170/80	64	Chronic nephritis and uremia
12	31	5.5	Phthalein trace	190/115	44	Pelonephritis
13	87	6.6	None recorded	190/90	58	Syphilis cardiac asthma chronic CV disease
14	38	4.0	None recorded	200/100	42	Chronic valvular disease, interstitial nephritis
15	87	7.0	None recorded (patient died soon after admission)	240/150	42	Chronic nephritis
16	69	4.3	Phthalein traces	160/100	60	Chronic nephritis
17	98	4.3	Phthalein traces	115/88	16	Chronic and subacute nephritis
18	38	4.0	None recorded (patient died soon after admission)	180/70	56	Chronic nephritis
19	66	3.2	None recorded	180/120	16	Chronic nephritis
20	38	3.2	Phthalein traces	152/72	36	Pelonephritis
21	11	1.0	Phthalein 90 per cent in two hr	100/60	3	Syphilis "CN" poisoning
22	45	5.0	Phthalein traces	190/70	44	Uremia polycystic kidney
23	41	4	Phthalein 10 per cent in two hr	200/120	4	Essential hypertension coronary sclerosis fibrillation and decompensation
24	45	4.0	Urea cone test normal		04	Uremia hypertrophied prostate

## POSITIVE DIAZO CASES

Of the thousand bloods which were examined, twenty four or 2.4 per cent were found to give a positive reaction. Of the twenty four cases, at least two failed to show a severe or even moderate degree of renal injury, as far as could be determined clinically or from laboratory tests. These two cases, 7 and 21 of Table I will be briefly described.

CASE 7—A S, aged seventy five admitted July 10, 1927

Chief Complaint—Headache, dizziness and spots before the eyes

Present Illness—The patient was well until one day prior to admission, when his vision became poor. Because of the above symptoms he was forced to go to bed.

*Physical Examination*—The patient's face was expressionless. The chest was emphysematous. The heart sounds were faint and he was in apparent mental confusion. The blood pressure was 164/84. The temperature was subnormal. Two days later a right-sided femoral pulse was noted and also signs of involvement of the eighth, ninth, and twelfth nerves.

*Laboratory Findings*—

Blood Chemistry Sugar 127, creatinin 14, urea 12, uric acid 6, mg per 100 cc

Blood Wassermann Negative

Urinalysis Negative for albumin and casts

Phthalein Test 21 per cent in two hours

Diazo Strongly positive on two successive examinations of the blood drawn at an interval of two days. The patient was discharged improved after two weeks in the hospital. A recent analysis of the blood (5 months after discharge) shows the following:

The diazo test negative, urea nitrogen 11.5 mg per 100 cc of blood, creatinin 1.6 mg per 100 cc of blood. The phthalein test shows an excretion of 60 per cent in two hours.

*Comment*—This case showed no retention of waste products in the blood throughout his stay in the hospital and at the present time of writing shows evidences of good renal function. He thus fails to confirm the opinion expressed by previous investigators that a positive diazo reaction is constantly associated with marked retention and severe renal insufficiency.

CASE 21—L. B., aged twenty-three, admitted November 15, 1927

*Chief Complaint*—The patient was brought into the hospital in semicomatose condition.

*Present Illness*—At 3 A.M. on the day of admission the patient had swallowed an unknown quantity of the commercial product "CN," after which she became unconscious. She was taken to the hospital very soon after where she quickly regained consciousness, but continued to vomit. She was lavaged and a hypodermoclysis was given after which she improved.

*Past History*—Scarlet fever at twelve years of age. There was a definite history of gonorrhea and syphilis. About five months prior to admission she claimed to have had pleurisy, nosebleeds and heart trouble. She stated that she had nocturia (twice a night).

*Physical Examination*—There was noted an irregularity and change in size of the right pupil. The rest of the physical examination showed no other essentially abnormal changes.

*Laboratory Findings*—

Blood Chemistry Urea N 11.0, creatinin, 1.9 mg per 100 cc

Blood Wassermann Positive

Urinalysis Negative

Phthalein Test 90 per cent in two hours

Diazo Test Strongly positive

The patient made an uneventful recovery and was discharged improved. Examinations subsequent to the discharge revealed nothing suggestive of renal disease. The diazo test was negative on the day of discharge and has remained negative up to date.

*Comment*—What has been said regarding the previous case also applies to this case. The blood chemistry is normal and the phthalein test output is high. It may be of interest to state that examination of a specimen containing "CN" failed to give a positive diazo reaction.

Further study of Table I throws some light on the question of the parallelism between a positive diazo and the phenolsulphonephthalein test on the creatinin content of the blood. Regarding the former, the table shows that although this kidney function test gave very low readings in the majority of the cases, two cases (8 and 23) show an excretion of 50 per cent each. It may

be added, however, that in both of these cases, the diazo tests became negative before their discharge from the hospital. As will be seen from the table, these cases were suffering from diseases not essentially renal. Case 8 was a diabetic who developed an urethral stricture complicated by pyelitis and cystitis. With effective drainage there was a synchronous decline of the waste products of the blood and a disappearance of the positive diazo reaction. Case 23 was essentially one of coronary sclerosis and thrombosis with auricular fibrillation and decompensation. The urea concentration of the urine was normal throughout and the high urea and creatinin of the blood dropped to normal with effective cardiac therapy. She is now reported doing very well.

Concerning the parallelism between the creatinin and the positive diazo test, a study of our cases again shows that a dogmatic statement cannot be made. The two cases already cited (Cases 7 and 21) each show a creatinin of 14 to 19 mg per 100 cc respectively, readings which are normal for our laboratory. It is to be noted that the cases which showed the greatest improvement were those whose blood creatinin was normal.

The question of prognosis as determined by the presence of a positive diazo test would perhaps best be postponed for discussion until a longer period of time has elapsed. It may be said however that at least several of our cases which showed a disappearance of the positive diazo at present show no indication of a progressing renal disease.

## NEGATIVE DIAZO TESTS

Table II shows a series of 14 cases in which the blood chemistry shows a definite or very marked retention of nitrogen but in which the diazo test

TABLE II  
NEGATIVE DIAZO WITH HIGH N.P.N. ETC

CASE NO	MG UREA N PER 100 CC BLOOD	MG CREATININ PER 100 CC BLOOD	RENAL FUNCTION TESTS	BLOOD PRESSURE	AGE	DIAGNOSIS
1	41.0	4.3	Phthalein trace	104/10	44	Hypertension cardiovascular disease
2	50.0	4.4	Indigo carmine test, no trace	170/30	4	Pyonephritis
3	52.0	4.2	Phthalein 30 per cent in two hr	180/80	26	Hypertension cardiovascular disease
4	49.4	3.0	Phthalein 16 per cent in two hr	220/14	3	Chronic nephritis general scler myocardial insufficiency
5	72.0	4.0	None recorded	138/12	60	Uremia
6	42.0	2.7	None recorded		44	Chronic myocarditis
7	31.0	2.7	None recorded	138/8	65	Coronary sclerosis cystitis
8	48.0	2.7	None recorded		64	Carcinoma of intestines
9	50.0	4.2	None recorded	80/50	45	Inguinal hernia
10	50.0	1.9	Phthalein 38 per cent in two hr		6	Cystitis pyonephritis
11	83.0	6.0	None recorded	90/60	3	Purpura sepsis nephritis (acute)
12	40.0	2.1	None recorded	90/60	44	Chronic myocarditis
13	33.0	3.0	None recorded	130/30	60	Cardiac decompensation
14	20.8	3.6	Phthalein no trace	110/100	6	Hypertrophied prostate



was repeatedly negative. In addition to high nitrogen retention, these cases also showed a low phenolsulphonephthalein excretion and in some there were clinical evidences of uremia.

#### SOME OBSERVATIONS OF PRACTICAL IMPORTANCE REGARDING THE TECHNIC

In the course of the examinations several observations have been made which may be considered of practical importance. The blood of markedly jaundiced individuals does not lend itself satisfactorily for the application of the diazo test. On addition of the sodium hydroxide a deep blue color appears which interferes with the pink color of a positive reaction. It is, therefore, frequently impossible to determine whether or not a jaundiced blood gives a positive diazo test. Another word of caution may be stated with regard to the application of the test to blood of a patient on whom a phenolsulphonephthalein test is being attempted. It has been observed that the appearance of a pink color after the addition of sodium hydroxide is, in such cases, sometimes due to the phenolsulphonephthalein present in the blood, thus disguising the pink color of a positive reaction. Lastly, it may be stated that the test does not necessarily have to be done immediately after securing the specimen of blood. Positive specimens have consistently given positive reactions even months after the specimens were obtained. The blood, however, must be kept in a cool place.

#### CONCLUSIONS

The following conclusions may be drawn from the above observations:

1. Although a positive diazo reaction on the blood serum or plasma is usually associated with a retention of waste products in the blood, it cannot be said that the association is a constant one, as has been shown from the study of Cases 7 and 21.

2. There is no constant parallelism between the phenolsulphonephthalein excretion and the presence of a positive diazo reaction. Two cases are cited in which the diazo test was positive in the presence of a phenolsulphonephthalein excretion of 50 per cent in two hours, and conversely, five cases are cited in which the nonprotein nitrogen of the blood is high and in which the phenolsulphonephthalein test is quite low and the diazo test is negative. The same applies to creatinin.

3. A positive diazo test, apparently, is not so ominous a prognostic sign if there are no other evidences of renal disease or blood nitrogen retention. The positive reaction in these cases may disappear and the patient may recover.

4. A combination of a positive diazo reaction and a high degree of creatinin retention is of greater prognostic significance than either one alone. The presence of both indicates a very poor prognosis.

5. Several observations are made which are of practical importance regarding the technic.

## REFERENCES

- <sup>1</sup>Andrews C H An Unexplained Diazo Color Reaction in Uremic Sera, *Lancet*, 1924, i 590  
<sup>2</sup>Becher, F Die Diazo und Urochromogen reaktion im Blutfiltrat bei Nieren Insufficienz ihre Erklärung und klinische Bedeutung, *Deutsch Arch f klin Med* 1925, cxlviii 10  
<sup>3</sup>Hewitt, L F The Diazo Reaction in Sera, *Biochem Jour*, 1925 xix, 171  
<sup>4</sup>Rabinowitch I M Observations on the Value of the Diazo Color Reaction in the Differential Diagnosis of Uremia *Canad Med Jour*, 1925, xxv 725  
<sup>5</sup>Blotner, H and Fitz R The Diazo Test in Nephritis *Jour Am Med Assn* 1927 lxxxiii 99,

## AUTOMATIC TEMPERATURE CONTROL FOR HOT AIR STERILIZERS IN THE BACTERIOLOGIC LABORATORY\*

BY IVAN C HALL PH D AND MARTHA I MILLER DENVER COLORADO

**A**LTHOUGH automatic devices for controlling temperatures in domestic gas ranges have been in use for several years so far as we know, the only bacteriologic hot air sterilizers in which the temperature may be accurately and automatically controlled are heated electrically but my somewhat limited experience with them has been unsatisfactory.

Without doubt, most of the older bacteriologic laboratories and many of the newer ones rely mainly upon the Lautenschlager type of gas operated oven for the sterilization of glassware, and there are probably few technicians who have not at one time or another burned up the cotton through their failure to watch the gas coal. Variation in gas pressure and lack of automatic control make the usual hot air sterilizer a matter of meticulous care and worry.

Three years ago at Cornell University we tried to secure the installation of a certain well known domestic gas oven regulator, only to be met with a proposal to discard our new hot air sterilizers in order to replace them with automatically controlled domestic ranges but this was inexpedient.

Subsequently the Robertshaw Thermostat Company of Youngwood Pennsylvania, placed upon the market a type of oven heat controller that may be easily installed in laboratory gas sterilizers, and we have had two of these in operation for about a year. The cost of each thermostat was ten dollars. The results have been entirely satisfactory from a practical standpoint but our study of their operation has raised some interesting points in the problem of hot air sterilization.

The detailed construction of the Robertshaw thermostat is shown in Fig. 1. The assembly is easily installed by a gas fitter who merely inserts the regulator into the gas line as shown in Fig 2, with the stem extending into the upper portion of the sterilizing chamber. In this figure, the shut off cock does not appear, it is located beneath the table.

After installation of the thermostat, the minimum burner pilot must be

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adjusted. The dial pointer is set at  $500^{\circ}\text{F}$ , the shut-off cock is opened, the gas burner is lighted in the usual way, and the oven is allowed to heat for about ten minutes. The pointer is then moved down to  $250^{\circ}\text{F}$ , closing the valve seat. Any gas then passing to the burner is through the minimum burner port and should give a flame about one eighth inch high over the entire burner. If the flame is too high, the screw (23) is turned to the right (clockwise) to reduce it, and if the flame is too low it is turned to the left.

The temperature setting also has to be regulated by turning the plug (22) at the end of the thermostatic tube. Turning the plug to the right (clockwise) raises the oven temperature, and to the left lowers it. The dial pointer (7) must be held in one position while the plug is being turned. If the plug is turned one face of its hexagon, it changes the temperature about  $50^{\circ}\text{F}$ .

Since the Robertshaw thermostat is now made mainly for domestic purposes, it uses the Fahrenheit scale. But as  $350^{\circ}\text{F}$  corresponds very nearly

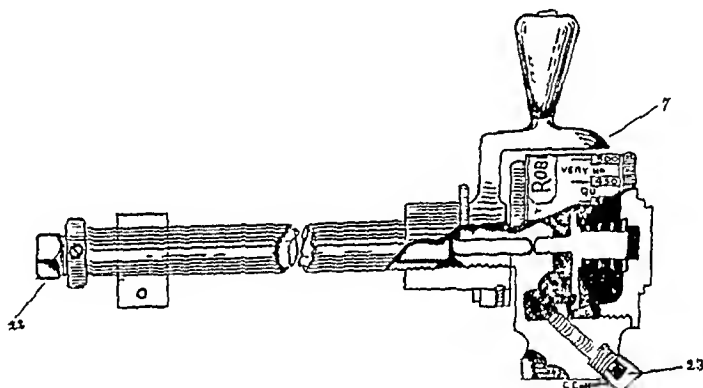


FIG 1—A cross section drawing showing the detailed construction of the Robertshaw Automatic Gas Thermostat.

to  $177^{\circ}\text{C}$  and the latter is quite satisfactory for the dry an sterilization of glassware, the dial pointer is set at  $350^{\circ}\text{F}$ . Other temperatures can be secured as desired. The dial pointer should be set before lighting the burner, it should never be changed while the sterilizer is hot.

With thermostatic control neither the beginning nor the close of sterilization differs in any way from the usual practice. The sterilizer should be loosely filled in order to insure circulation and penetration of heat. When the hour is concluded, the gas is turned off at the cock in the usual manner, and the contents allowed to cool somewhat before removal, in order to avoid breakage from cold drafts. The only difference is that absolutely no attention is required during sterilization, and the advantage of this needs no emphasis.

In order to display numerical data on the operation of our hot air sterilizers with automatic control, we performed numerous experiments, of which only the most significant ones are described.

Using at first a single thermometer in each sterilizer, we soon learned

that if readings were made at five minute intervals after lighting the burners the temperature rose rapidly to several degrees above the desired heat, but quickly dropped, and thereafter remained constant indefinitely and automatically. This "period of lag" in the stabilization of the temperature is no doubt, due to the time required to heat the regulatory apparatus itself.

Since each sterilizer has two ports in the top, one on each side of the central vent, we decided to supply each of these with a tested thermometer, as an additional check upon the accuracy of our results. To our great surprise it was found that in each sterilizer one thermometer always read higher by several degrees than the other. In some cases the

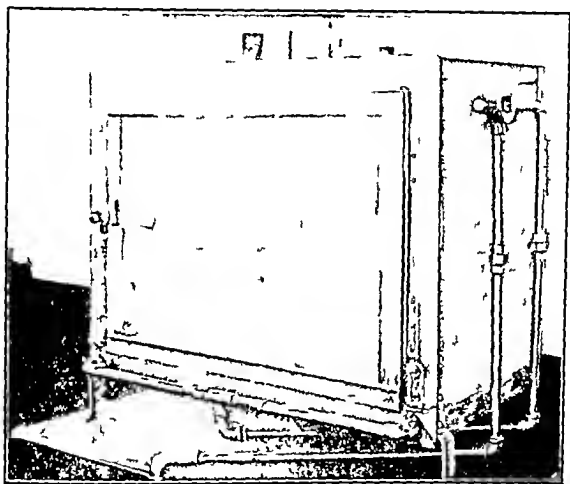


Fig. —An old hot air sterilizer equipped with automatic temperature control

differences amounted to as many as  $40^{\circ}\text{C}$ , in a few instances they were not more than  $4$  or  $5^{\circ}\text{C}$ , but often they were  $15$  to  $25^{\circ}\text{C}$ . This was disconcerting and we undertook to find the reason for it since, although we had not experienced any practical difficulty in sterilization, it was apparently possible for some objects in the sterilizer to be heated at a much lower temperature than we desired even when another portion approached a heat that would scorch paper.

In the beginning, the two sterilizers were mounted separately and the gas supply connected diagonally as in Fig 2. At a later time, it became expedient to locate the two sterilizers side by side in a ventilated hood. Suspecting that the diagonal direction of the gas supply was responsible for a hotter flame on one side of the sterilizer than on the other the piping was

changed so as to enter the T-branch supplying the burners at a right angle. This did not affect the inequality of the thermometer readings, however.

We tried operating the sterilizers empty, loaded, singly, and simultaneously, taking temperatures every five minutes from the beginning. Running both sterilizers at the same time, the one on the left (A) showed a constant difference after stabilization of  $40^{\circ}\text{C}$ , one thermometer reading  $186^{\circ}\text{C}$  while the other read  $146^{\circ}\text{C}$ . At the peak of the temperature curve just prior to stabilization the readings were  $151^{\circ}\text{C}$  and  $196^{\circ}\text{C}$ , a difference of  $45^{\circ}\text{C}$ . The sterilizer on the right (B), gave readings at the peak before stabilization of  $205^{\circ}\text{C}$  and  $191^{\circ}\text{C}$  (difference,  $14^{\circ}$ ) but after stabilization,  $185^{\circ}\text{C}$  and  $175^{\circ}\text{C}$  (difference,  $10^{\circ}$ ). In both sterilizers, the adjacent sides gave the higher readings, suggesting an influence of each upon the other, for the walls were only separated by 10 cm.

When the sterilizers were operated empty and singly, the differences in temperature readings were less but still in the same direction, "A" gave constant readings after stabilization of  $154^{\circ}\text{C}$  and  $178^{\circ}\text{C}$ , a difference of  $24^{\circ}\text{C}$ , while "B" gave constant readings of  $182^{\circ}\text{C}$  and  $178^{\circ}\text{C}$ , a difference of only  $4^{\circ}\text{C}$ .

When the sterilizers were operated under load (baskets filled with plugged test tubes), "A" showed constant readings after stabilization of  $170^{\circ}\text{C}$  and  $195^{\circ}\text{C}$ , a difference of  $25^{\circ}\text{C}$ , while "B" showed constant readings of  $184^{\circ}\text{C}$  and  $168^{\circ}\text{C}$  a difference of  $16^{\circ}\text{C}$ .

Various adjustments were tried to equalize the temperature on both sides. We found that the damper in the main vent at the top must be left open because the gas flame was smothered out if it was closed. The side ports, used for lighting the flame, on the other hand, might be left open or closed without materially affecting the temperature readings on the operation of the sterilizers. Finally, we found on sterilizer "B" that by adjusting the air mixer ports one at a time after the temperatures had become stabilized we could equalize the two sides to within  $1^{\circ}\text{C}$ . On this sterilizer, the left side had always operated at 4 to  $16^{\circ}\text{C}$  higher than the right side. With constant reading after stabilization, in a certain experiment, of  $181^{\circ}\text{C}$  and  $176^{\circ}\text{C}$  (difference,  $5^{\circ}\text{C}$ ), the right air mixer was opened wide, this increased the reading on the left side in twenty minutes to  $186^{\circ}$  and decreased that on the right side to  $173^{\circ}\text{C}$  (difference,  $13^{\circ}$ ). Then the left air mixer port was opened wide while the right was left about three-fourths closed, in twenty minutes the reading on the left was  $176^{\circ}\text{C}$  while that on the right was  $175^{\circ}\text{C}$ , and these temperatures were maintained constantly for fifty minutes when the experiment was concluded.

A similar experiment was then performed on sterilizer "A" with essentially similar results, it was possible by adjusting the air mixer ports to stabilize and equalize the left and right thermometer readings at  $183^{\circ}\text{C}$  and this could be maintained indefinitely without attention. This was a little too high but the temperature was easily reduced by means of the plug (22). The sterilizer was then allowed to cool and a final demonstration run made with a comfortable load of plugged test tubes, data for which, are

shown graphically in Table I which displays (a) the somewhat uneven rise on the two sides of the chamber, (b) the humps in the temperature curves preceding stabilization, (c) the accurate maintenance of a constant temperature ( $175^{\circ}\text{C}$ ) on both sides for one hour, and (d) the slight effect on sterilizer "A," of starting sterilizer "B" at the end of the hour

TABLE I

THERMOMETER READINGS SHOWING THE CONSTANT TEMPERATURES MAINTAINED BY THE AUTOMATIC HEAT CONTROLLER AND THE SLIGHT INFLUENCE OF STARTING AN ADJACENT STERILIZER

TIME IN MINUTES	STERILIZER A		STERILIZER B	
	LEFT	RIGHT	LEFT	RIGHT
0	24	24		
5	47	48		
10	85	85		
15	130	136		
20	160	162		
25	160	175		
30	178	184		
35	180	185		
40	168	183		
45	175	180		
50	175	175		
55	175	175		
60 (1 hour)	175	175		
65	175	175		
70	175	175		
75	175	175		
80	175	175		
85	175	175		
90	175	175		
95	175	175		
100	175	175		
105	175	175		
110	175	175		
115	175	175	37	34
120 (2 hours)	175	175	7	7
125	175	175	120	142
130	175	175	152	180
135	175	177	164	185
140	175	177	173	173
145	175	179	174	173
150	175	177	175	173
155	175	177	175	173
160	175	175	175	173

Degrees Centigrade

It is interesting in connection with the last point (d) to note that the difference in reading of the two thermometers in "A" was about twice as great ( $4^{\circ}\text{C}$ ) during the peak of the curve for "B" as after restabilization ( $2^{\circ}\text{C}$ ). The influence of one sterilizer upon the adjacent one is seen to be essentially negligible.

Our hot air sterilizers have no means for controlling or regulating the amount of gas passing to either side of the ovens, it is evident that the lack of this adjustment accounts for the surprising differences that we first found in the two sides of our sterilizers. We are aware, of course that more modern models of hot air sterilizers are properly equipped in this regard.

The air mixer adjustment on our old sterilizers is also of the crudest possible type, consisting merely of sliding sleeves over holes in the burner pipes. We have called attention to these points because we suspect that many bacteriologic laboratories are equipped with hot air sterilizers no better than ours and it may be that some of the contaminations encountered by even bacteriologists, are attributable to ineffective hot air sterilization due to variation in temperature, such as we have described, although we ourselves have had no evidence of difficulty attributable to this cause. At any rate, the automatic control is thoroughly worth while.

#### SUMMARY

- 1 A type of automatic domestic gas range thermostat which maintains constant temperatures indefinitely is advocated for hot air sterilizers
- 2 Attention is called to the necessity of suitable adjustments to insure uniform temperature inside such sterilizers
- 3 The results of various conditions of operation of hot air sterilizers are noted

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## BLOOD GROUPS

### THE NEED OF UNIFORMITY OF TERMINOLOGY IN CLASSIFICATION\*

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AT THE present time three methods of classifying the four basic human blood groups are in use, namely, the numerical classifications of Jansky and Moss and the International Classification which uses letters. The need, therefore, of a uniform terminology is quite apparent.

Uniformity of terminology can be obtained by the medical profession by the universal adoption of any one of these three classifications. However, it is felt that the following brief history and other data pertaining to blood grouping will show why the adoption of the international nomenclature is preferable as a logical and tactful solution of the problem.

In 1901, Landsteiner established the principle of blood grouping when he discovered the existence of three of the four basic human blood groups. He classified these three groups by the letters A, B, and C. Subsequently several workers, among them von Decastello, Sturli, and Hektoen, found exceptions to these original three groups.

However, it was left to Jansky, in 1907, to first incorporate these exceptions into a fourth group. His classification of the four groups was numerical, namely, I, II, III, and IV. This work was published in the Bohemian

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language, and apparently failed to arouse interest owing to obscurity and inaccessibility of the publication

Therefore, in 1910, when Moss, an American reported findings similar to Jansky's, it was not unnatural that his classification took precedence over Jansky's in at least the English speaking countries. There is no question but that Moss worked independently and without knowledge of Jansky's findings. Moss likewise used a numerical classification but perhaps unfortunately, his groups I and IV were just the reverse of those of Jansky's. The result has been that in communities where the two classifications are used there is considerable confusion and at times actual danger.

In order to avoid this confusion and danger, the American Association of Immunologists, the American Medical Association, the Society of American Bacteriologists and the Association of Pathologists and Bacteriologists, the Medical Department of the U S Navy, and others in 1921 adopted on the basis of priority Jansky's classification. That this classification has priority over Moss's is beyond controversy. In fact appended to Moss's article is a statement by Moss to the effect that since his paper went to press Jansky's work was brought to his notice and that had he known about it in time he would have given Jansky credit for priority in establishing the correct classification.

Unfortunately many organizations and individuals in this and other countries did not give preference to Jansky's classification. Therefore, the confusion and danger still exist.

Last year, 1927, in the interest of uniform terminology and in an attempt to break what might be called the "Moss-Jansky deadlock" the American Association of Immunologists adopted what tactfully should be called the International Classification. The various other names used for this classification are Landsteiner, Modified Landsteiner, National Research Council Classification etc. The name of this new letter nomenclature however is quite secondary. The idea originated with the discovery of the groups, Landsteiner, and has the approval of the National Research Council and the medical authorities of many European countries. This classification is now the official method of reporting blood groups in the Medical Department of the U S Army and the U S Navy.

The International Classification uses the letters O, A, B and AB, which designate directly von Dungern and Hirschfeld's hypothetic agglutinin content of the red cells.

According to von Dungern and Hirschfeld's hypothetic explanation, there is distributed among the red blood cells of the groups two agglutinogens designated "A" and "B". Neither agglutinin is present in group "O" ("O" could really mean "Zero"). Agglutinin "A" is present in group "A". Agglutinin "B" is present in group "B". Both agglutinogens "A" and agglutinin "B" are present in group "AB".

Table I compares the International, Jansky and Moss Classifications and also shows the compatibility of the red blood cells with the sera of the various groups.



TABLE I  
INTERNATIONAL, JANSKY, AND MOSS CLASSIFICATIONS COMPARED

CFLS OF GROUPS			SERUM OF GROUPS			
			O I IV	A II II	B III III	AB IV I
†	*	∖				
O	I	IV	—	—	—	—
A	II	II	—	—	+	—
B	III	III	+	+	—	—
AB	IV	I	+	+	+	—

† Letters indicate International Classification

\* Numbers indicate Jansky Classification

∖ Numbers indicate Moss Classification

— Indicates no agglutination

+ Indicates agglutination

#### INTERNATIONAL CLASSIFICATION—DONOR (CELLS)

*Group O*—Red blood cells contain no agglutinogens and are therefore not agglutinated by sera of any group. Members of this group are known as "universal donors."

*Group A*—Red blood cells contain agglutinogen A and are not agglutinated by sera of Groups A or AB. In other words no agglutination with sera of groups showing the letter A.

*Group B*—Red blood cells contain agglutinogen B and are not agglutinated by sera of Groups B or AB. In other words no agglutination with sera of groups showing the letter B.

*Group AB*—Red blood cells contain agglutinogens A and B in combination and are not agglutinated by the serum of Group AB. In other words no agglutination with the one group showing letters AB in combination.

#### INTERNATIONAL CLASSIFICATION—RECIPIENT (SERUM)

*Group O*—Serum will not agglutinate cells of Group O.

*Group A*—Serum will not agglutinate cells of Group A or Group O.

*Group B*—Serum will not agglutinate cells of Group B or Group O.

*Group AB*—Serum will not agglutinate cells of any group. Members of this group are known as "universal recipients."

#### RESUME

Group O donor can give blood to a recipient of group O, A, B, or AB

Group A donor can give blood to a recipient of group A or AB

Group B donor can give blood to a recipient of group B or AB

Group AB donor can give blood to a recipient of group AB

Group O recipient can receive blood from donor of group O

Group A recipient can receive blood from donor of group O or A

Group B recipient can receive blood from donor of group O or B

Group AB recipient can receive blood from donor of group O, A, B, or AB

From the foregoing it will be readily seen that the International Classification differs from the Jansky and Moss classifications merely in the substitution of the letters O, A, B, and AB, for the numbers I, II, III, IV and IV, II, III, I, respectively. It will likewise be noted that it is only necessary to have on hand group A and group B sera in order to determine the group to which the blood of any individual belongs.

In typing bloods it is important to remember that there are atypical groups. Therefore in addition to grouping donor and recipient, direct matching of the cells and sera of the donor and recipient should be made a routine procedure.

In conclusion it may be said that some of the points in favor of the adoption of the International Classification are

1 It appears to offer a logical and tactful solution in securing an international uniform terminology

2 It has a more scientific basis

3 The compatible blood groups are more easily remembered, which incidentally facilitates teaching

4 No change in principle is required, merely substituting letters for numbers

5 It is the idea of the discoverer of the principle of blood grouping and its adoption therefore would really be in his honor

#### REFERENCES

- Landsteiner K Zentralbl Bakteriol 1909 xxvii 361 Wien klin Wchnschr, 1901 xiv, 1152  
 Decastello A von and Starh A Munchen med Wchnschr 1902 xlv 1090  
 Hektoen L Jour Infect Dis, 1907, iv 29, Jour Am Med Assn 1907, xlviii 1739  
 Jansky J Klinicky Sbornik 1907 viii 85  
 Moss W L Trans Assn Am Phys 1909, xxiv 419, Johns Hopkins Hosp Bull, 1910, xxi 63  
 Dungren E von and Hirschfeld L Ztschr Immunitatsf 1910, vi 284, 1911, viii, 527  
 Jour Am Med Assn Jan 8 1921 lxxvi 139  
 Doan C A Physiological Reviews 1927 vii 1  
 Jour Am Med Assn April 30 1927 lxxviii 1421  
 Stitt E R Practical Bacteriology, Blood Work and Parasitology, 1927 ed 8 p 329  
 Circular letter Serial No 461, Medical Department U S Navy Feb 28, 1928  
 Circular letter No 7, Medical Department U S Army, March 5, 1928

### PRACTICAL USES OF HEPARIN\*

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IT IS the purpose of this paper to bring to the attention of those interested certain practical uses of heparin suggested by actual experience extending over several years. Howell and Holt<sup>1</sup> first described this substance and the senior author has since published several communications<sup>2, 3</sup> on its chemical nature and reactions, and quite recently has described<sup>4</sup> a method of purifying heparin to a high degree.

Mason<sup>5a</sup> has employed this material in intravascular experiments on animals and also has employed it in transfusion in 33 human subjects. In some cases subjective symptoms occurred suggestive of toxicity from impurities. He has further suggested its use in prevention of thrombosis.<sup>5b</sup> This work did not come to our attention until quite recently. Independently and without knowledge of Mason's experience, heparin was employed intravascularly in about 200 experiments on dogs (Reed).

Following its successful intravascular use<sup>5</sup> in etherized dogs, it seemed possible that it might be used in blood for transfusion purposes. In a per-

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sonal communication to me in 1925, Prof Howell suggested certain lines of investigations that should precede any attempt to employ it on human patients. These proved negative. It is not necessary to describe the experiments in detail. It is sufficient to state that in both normal and etherized animals (dogs and rabbits) heparin in concentrations up to 60 mg (crude commercial preparation) per kilo of body weight has never been found to affect in any way the body temperature, the force or rate of the heart, blood pressure, concentration of blood cells, respiration rate or volume, salivary secretion, activity of smooth muscle, or neuromuscular excitability to galvanic stimulation when given in single large doses. No efforts have been made to study its effects on the kidney.

In one paper,<sup>2b</sup> Howell suggested that heparin in the form then obtainable contained some nitrogen, though the more highly purified form now obtainable contains no nitrogen or phosphorus. Prompted by the earlier report Reed and Lamson<sup>6</sup> decided to investigate the possibility of sensitization of guinea pigs. With one lot of heparin a few animals displayed symptoms suggestive of anaphylaxis. But subsequent similar tests with other lots proved entirely negative so that it can be stated that neither toxic nor allergic symptoms result from repeated doses to guinea pigs. These negative results are readily explained by Howell's recent findings that this substance is a carbohydrate body, probably a derivative of glucuronic acid.<sup>2</sup>

Howell has classified this substance as an antipiothrombin. In very small amounts, depending on the extent of purification, it will inhibit clotting of blood until hemolysis occurs. And it has been determined<sup>7</sup> that its inhibitory power is still effective when added some time after the blood is drawn. In some cases there was complete inhibition when heparin was added within thirty seconds of the termination of clotting time as determined on normal blood. From this it is apparent that the particular point at which heparin is effective occurs quite late in the total process of clotting.

On account of the relation of clotting to anaphylactic symptoms<sup>8</sup> a number of investigations have been undertaken to discover whether heparin modifies these symptoms. Keyes and Strauser<sup>9</sup> and Van de Carr and Williams<sup>10</sup> have found that in pigeons and guinea pigs sensitized to horse serum heparin prevented or modified the symptoms of anaphylaxis produced by a "shock" dose of horse serum. Hanzlik, Butt and Stockton,<sup>11</sup> Hyde,<sup>12</sup> Waud,<sup>13</sup> and Reed and Lamson<sup>14</sup> have failed to demonstrate such modification or protection. This controversy, however, belongs to another field and need not be discussed further here. But it is now certain that there are no *harmful* results from use of heparin in this connection.

During the past three years a large number of blood analyses have been made in this laboratory in which blood samples were heparinized. Extensive comparisons with other methods of preventing clotting indicate that the presence of minute amounts of heparin required for this purpose does not interfere with any routine chemical determination. If very large amounts are used it may interfere with colorimetric readings but there is no occasion for use of such quantities. Furthermore, intravascular injections of heparin do not modify any blood constituent ordinarily determined in laboratory work (inorganic salts, sugar, formed elements).

Pantos and Svec<sup>13</sup> have claimed that any agency increasing clotting time lowers blood sugar. This has not been confirmed in our experience with heparin.

Rowntree and Shionova<sup>14</sup> have employed heparin in a study of the mechanism of thrombus formation and have found that when the blood was circulated through a collodion tube, a white thrombus soon formed, even with large single doses of heparin, whenever there was any irregularity of the surface. This demonstrates the inhibition of fibrin formation.

If blood is drawn rapidly, it may be heparinized after removing from the syringe. But if it must be drawn slowly, 1 or 2 mg of heparin may be placed in the dry syringe. Because of the slow solubility of heparin, however, it has been found to be advantageous to moisten it with a minute drop of salt solution.

Professor Howell's latest paper contains a report of eight successful transfusions made in human patients and two others in which there was an elevation of temperature in all of which 1 mg of heparin was added to each 100 cc of blood. Such reactions have not been found in dogs or rabbits in this laboratory.

It appears then that heparin is a highly specific substance which is remarkably inert in relation to all other physiologic functions so far studied.

#### SUMMARY

Heparin is a specific substance which while expensive as now supplied promises to be of great value in scientific investigations.

1 It may be used successfully in animal experiments where it is desired to retain or restore intravascular fluidity, without modifying in any way other functions.

2 It is of value in preserving fluidity of blood samples for chemical analyses since it does not modify the chemical content of the more commonly determined constituents.

3 It has been successfully employed clinically in transfusions and all evidence available at present indicates that it may be so employed with safety.

#### REFERENCES

- <sup>1</sup>Howell and Holt. *Am Jour Physiol* 1915 *xlvii*, 325
- <sup>2</sup>Howell (a) *Ibid*, 1922, *lxxii*, 434 (b) 1925 *lxxi*, 653
- <sup>3</sup>Howell. *Bull Johns Hopkins Hosp*, 1928 *xl*, 199
- <sup>4</sup>Mason (a) *Surg Gynec Obst* 1924 *xxviii*, 491 (b) *JOUR LAB AND CLIN MED* 1925, *x*, 203
- <sup>5</sup>Reed. *Am Jour Physiol* 1925, *xliv*, 79
- <sup>6</sup>Reed and Lamson. *Proc Soc Exper Biol and Med* 1926, *xxiii*, 6, 6
- <sup>7</sup>Reed. *Am Jour Physiol*, 1926, *lxxvii*, 568
- <sup>8</sup>Cahring. *Jour Immunol*, 1926, *xi*, 477, Biedl and Kraus. *Wien klin. Wchnschr* 1909 *vi*, 363, Zinnser, *Infection and Resistance*, 1923, 436 New York. Friedberger. *Ztschr f Immunitätsf*, 1909, *iv*, 636
- <sup>9</sup>Keyes and Strauser. *Jour Immunol* 1926 *xii*, 419
- <sup>10</sup>Van de Carr and Williams. *Proc Exper Biol and Med* 1927, *xxiv*, 798. *Jour Immunol* 1927, *xv*, 12
- <sup>11</sup>Hanzlik, Butt and Stockton. *Jour Immunol* 1927 *xiii*, 409
- <sup>12</sup>Hyde. *Am Jour Hyg* 1927, *vii*, 614
- <sup>13</sup>Waud. *Am Jour Physiol*, 1927, *lxxxv*, 160
- <sup>14</sup>Reed and Lamson. *Jour Immunol* 1927, *xiii*, 433
- <sup>15</sup>Pantos and Svec. *Deutsch Med Wchnschr* 1927 *liii*, 1857
- <sup>16</sup>Rowntree and Shionova. *Jour Exper Med*, 1925 *x*, 203

# A COMBINED PEROXIDASE AND WRIGHT'S STAIN FOR ROUTINE BLOOD SMEARS\*

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**D**URING the past five years various peroxidase reagents have been used by me in attempting to differentiate clearly between the blood cells of lymphocytic and those of myelocytic origin. It was desired to find a method of staining which would demonstrate in a clear-cut way not only the presence or absence of the peroxidase granules but also the essential characteristics of each cell as usually shown by a simple Wright's stain. This would make it possible to determine the distribution of peroxidase granules in each individual cell-type rather than merely the percentage of leucocytes showing granules. In addition it would enable the examiner to do a routine differential white blood count at the same time. To be of value for routine laboratory work, such a staining method should be easily performed and not too time-consuming. Moreover, the stains used should be stable enough so that good results could be obtained for at least six months.

It is not the purpose of this paper to go into any discussion of the chemical reactions involved in the peroxidase reaction. Nor is it concerned with the exact value or limitations of peroxidase stains in general. The stains described by Graham,<sup>1 2</sup> McJunkin,<sup>3</sup> Goodpasture<sup>4</sup> and Sato<sup>5</sup> have all been used by me with more or less satisfactory results as far as a demonstration of the peroxidase granules is concerned, but no one of them has fulfilled all the desired results described in the preceding paragraph. It is, then, the purpose of this paper to describe a new method for obtaining such results. It should be noted that no materials are used which have not been previously described by Graham, McJunkin or Goodpasture, only the methods of preparation and staining have been altered.

## STAINS

*Solution No. 1*—This first stain is a modification of Goodpasture's and contains

Benzidine Base	0.3 gm	
Basic Fuchsin	0.3 gm	
Sodium Nitroprusside	1.0 cc	(saturated aqueous sol.)
Ethyl Alcohol (95 per cent)	100.0 cc	

The benzidine and fuchsin are dissolved in the alcohol in the order named. Then the saturated solution of sodium nitroprusside is added. A slight precipitate may form at the bottom of the flask but does not interfere with the efficacy of the stain. This solution will give consistently good results for eight

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to ten months (I have obtained satisfactory stains from one lot which had been kept in a plain glass dropping bottle for more than a year)

*Solution No 2*—This second solution is also a modification of Goodpasture's stain, containing

Hydrogen Peroxide	5 to 6 drops
Tap Water	25 cc

In order to obtain the best results this solution should not be over forty eight hours old, but it will give fairly good results for three to four weeks. The ease with which it is made up offsets the possible disadvantage of so unstable a solution. The hydrogen peroxide itself if originally fresh, will keep for about six months in a dark, well stoppered bottle.

*Solution No 3*—Ethyl Alcohol (95 per cent)

*Solution No 4*—Wright's Stain

The variations so frequently observed in different lots of Wright's stain, when it is used for routine blood smears, become much more pronounced when it is used as a counter stain. I have tried out many different lots of powdered Wright's stain both as made up by technicians and in my own laboratory, but the results have been exceedingly uncertain. Therefore, it seems safer to advise the use of a Wright's stain which is made up ready for use by some reliable commercial firm. (The stains to be described in this paper were all made with that of the National Aniline and Chemical Co., Inc. New York.)

*Solution No 5*—Water

Tap water has been used with most satisfactory results in such widely separated places as New York, Massachusetts, Oregon and California. Freshly distilled water may be substituted if desired but has no particular advantages. Distilled water which has stood for some days in the laboratory is distinctly less reliable than tap water.

#### METHOD OF STAINING

In order to obtain the clean cut stain described below the following procedures must be carried out with reasonable exactness.

1 Smears should be made preferably on cover slips, thinly spread and allowed to dry in the air. They should be stained within three to four hours if possible since the peroxidase reaction cannot be relied upon in smears over twelve hours old unless they have been well protected from light and air. (The subsequent directions are for cover slip smears.)

2 The cover slip is flooded with 10 drops of solution No 1 and allowed to stand one to one and one half minutes.

3 Five drops of solution No 2 (about one half the quantity of No 1) are added to the cover slip without pouring off the No 1 solution, and allowed to stand three to four minutes.

4 The slip is then washed thoroughly in tap water (one half to one minute).

5 While still wet it is flooded with 95 per cent alcohol and allowed to stand three to four minutes or until completely decolorized (i.e., when there is no more pink visible to the naked eye).

6 It is then washed thoroughly under the tap and dried

7 The cover slip is then flooded with 8 drops of solution No 4 (Wright's stain) and allowed to stand for two to three minutes

8 Twelve to 14 drops of tap water (one and one-half times as much water as Wright's stain) are added and allowed to stand for twenty to forty-five minutes (Most normal and many abnormal bloods will stain well in twenty to twenty-five minutes but certain abnormal bloods, particularly the leucemic bloods, require thirty-five to forty minutes)

9 The cover slip is washed briefly under the tap, flooded with 95 per cent alcohol for three to five seconds and immediately washed under the tap for ten to fifteen seconds

10 It is then dried and mounted

The experiment of placing this stain in the hands of medical students and interns has resulted in the knowledge that good stains may be obtained only by adhering quite closely to the above directions

#### DESCRIPTION OF THE RESULTING STAIN

When the above mentioned procedures have been faithfully carried out there results a stain which shows both the peroxidase granules and most of the salient characteristics of the various cells which may be seen with Wright's stain alone. The following description of different cell-types is based upon examinations of several hundred smears taken from normal bloods as well as from patients suffering from a great variety of complaints. Most of the patients were infants, children, and young adults. The diseases studied included acute and chronic infections with and without leucocytosis, metabolic disturbances, secondary anemias of all kinds, leucemias with and without leucocytosis, neoplasms, purpuras, hemophilia, hemorrhagic disease of the newborn, malaria, one encephalitis, one aplastic anemia, and one chloroma. The blood of both normal and premature newborn infants has also been examined. Consequently there has been ample opportunity to observe almost every kind of cell which may appear in the circulating blood.

As a control, smears taken at the same time, and stained with Wright's stain alone, have always been compared with those stained with the combined peroxidase and Wright's stains. In addition a good many of the abnormal bloods have been examined at the same time with vital staining of fresh preparations, using neutral red (Grubler) and Janus green (National Aniline and Chemical Co). This latter procedure has proved of especial value in differentiating between the various mononuclear cells, i.e., lymphocytes, monocytes, and endothelial phagocytes.<sup>6</sup>

The normal polymorphonuclear neutrophil is so full of the large black peroxidase granules that frequently the characteristic pleomorphic purple nucleus is the only other element visible. But in the few polymorphonuclears which usually show a smaller number of the black granules, the cytoplasm may be made out as finely granular and either neutrophilic or slightly pinkish. In certain abnormal bloods, notably in the myelogenous leucemias, some of these cells may have very few or no peroxidase granules. The cytoplasm of these cells frequently appears to be vacuolated, suggesting that they

are degenerate forms in which the substance producing the peroxidase reaction has already disappeared

The eosinophils are equally characteristic. Their nuclei stain the usual purple, as with Wright's stain, and the large eosinophilic granules take on the deep black of the peroxidase stain but remain refractile as always. This gives them the appearance of very large black granules whose center is slightly paler than the periphery and of a brownish tint.

The basophils are the only normal cells which are hard to classify with this stain, since the basophilic granules take on the black of the peroxidase reaction so that their differentiation from neutrophils is difficult. But the granules are slightly larger and tend to be arranged more thickly at the cell edges. The nucleus is also to be distinguished as less pleomorphic and of a paler purple or even lavender color, compared with that of the neutrophil.

The lymphocytes never show any peroxidase granules. Their characteristics by this staining method are identical with those seen in a good Wright's stain.

The endothelial type of large mononuclear cell usually shows a scattering of black granules which tend to be present largely in groups. The remainder of the cell stains as with Wright's stain alone. That is, the nucleus is a slightly paler shade of purple than that of the neutrophils or lymphocytes while the cytoplasm shows a slate grey homogeneous background with fine pinkish granulation, unevenly distributed. Occasionally one sees these cells with only one or two or even without any peroxidase granules. This finding was noted particularly in two cases which showed abnormally large numbers of these cells in the circulation. The suggestion is made that this type of cells shows granules only when material which contained peroxidase has been phagocytosed.

The large mononuclear cells which are neither lymphocytes nor endothelial cells invariably show few to many peroxidase granules. These are best described as monocytes. Their peroxidase granules usually appear smaller than those in the neutrophils and more diffusely distributed throughout the cell than is the case with the endothelial type of mononuclear. The nucleus and cytoplasm stain as with a simple Wright's stain. That is, the nucleus is purple and the cytoplasm very finely granular, giving the impression of a homogeneous blue grey until one examines it closely enough to observe the presence of granulation. Occasionally these cells, like the neutrophils, are so full of black granules that their cytoplasm is obscured.

Myelocytes usually show some peroxidase granules. The number may vary from 2 or 3 to a score or more so that the cell is as full of granules as a mature neutrophil. The myelocytes which are less granular by Wright's stain show fewer peroxidase granules. In cases of acute myelogenous leucemia, when the blood stream is flooded with myelocytes in all stages of maturity with eosinophilic, basophilic and neutrophilic granulation, the peroxidase granules may be less clear cut than normally. But there is no difficulty in determining their presence.

Myeloblasts and lymphoblasts never show any peroxidase granules so that their differentiation is no more possible with this stain than with any



other, if a difference actually exists. Their appearance is identical with that seen in Wright's stain alone.

However, it is worth remembering that some of the large primitive leucocytes with deep blue cytoplasm and large round purple nuclei may be very early myelocytes whose granules are too few and small to be picked up with an ordinary Wright's stain. Such cells will show a few black granules with the peroxidase stain, thus being marked as of myelogenous origin. In a case of leucemia recently seen by the author such a finding was of definite value in arriving at a diagnosis of myelogenous, rather than lymphatic, leukemia.

The normal erythrocytes stain a greyish or brownish color with a tint of pink. The shade may be varied considerably by increasing or decreasing the length of the final decolorization process. (See "Method of Staining," Sec 9.) Basophilic stippling and polychromatophilia may be demonstrated as clearly as with Wright's stain alone. On several occasions peroxidase granules have been demonstrated in immature nucleated red blood cells, but this is not the usual finding. Malarial parasites are slightly less visible than with Wright's stain alone.

The platelets stain exactly as with a simple Wright's stain. They never show any peroxidase granules.

#### SUMMARY

A new method has been described for the demonstration of the peroxidase reaction in white blood cells.

The process consists of three steps: staining with a modification of Goodpasture's stain, decolorizing with 95 per cent alcohol, and counterstaining with Wright's stain.

In modifying Goodpasture's stain a solution has been obtained which will remain stable for eight to twelve months.

A detailed description of the resulting stain is presented.

By this method one may obtain a clear-cut picture of the peroxidase content of each cell without interfering with their routine classification as with Wright's stain alone.

#### REFERENCES

- <sup>1</sup>Graham, G. S. The Oxidizing Ferment of the Myelocytic Series of Cells and Its Demonstration by an Alpha naphthol pyronin Method, *Jour Med Research*, November, 1916, **xxv**, 231-242.
- <sup>2</sup>Graham, G. S. Benzidine as a Peroxidase Reagent for Blood Smears and Tissue, *Jour Med Research*, September, 1918, **xxv**, 15-24.
- <sup>3</sup>McJunkin, F. A. A Benzidine Polychrome Stain for Blood, *Jour Am Med Assn*, Jan 3, 1920, **lxiv**, 17.
- <sup>4</sup>Goodpasture, E. W. A Peroxidase Reaction with Sodium Nitroprusside and Benzidine in Blood Smears and Tissues, *Jour Lab and Clin Med*, 1919, **iv**, 442.
- <sup>5</sup>Sato, A., and Yoshimatsu, S. Peroxidase Reaction in Epidemic Encephalitis, New Diagnostic and Prognostic Methods, *Am Jour Dis Child*, March, 1925, **xv**, 301.
- <sup>6</sup>Simpson, M. E. The Experimental Production of Macrophages in the Circulating Blood, *Jour Med Research*, April-May, 1922, **xlvi**, 77-144.

# ACCURATE COLORIMETRIC TECHNIC FOR BLOOD AND TISSUE CHOLESTEROL ESTIMATIONS\*

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## BLOOD CHOLESTEROL

THE dependability of any colorimetric method for blood cholesterol may be judged, in a practical way, from the closeness of check determinations on samples of a given specimen of blood. Where absolute accuracy is necessary one must of course be sure that extraction is complete. It is surprising though, how difficult it is to obtain accurate checks with such thoroughly good methods as those of Bloor,<sup>1</sup> Sackett, Myers and Wardell,<sup>2</sup> Leboff,<sup>3</sup> through no fault of the methods, but rather because of incomplete control of the technic.

I find that among the important points in technic, the nature of the extraction, the volumes of solvents, the nature of the standard solution, are all deserving of special care, while for the colorimetric comparison of specimens and standard samples one must standardize very accurately the factors of light temperature and maturation time.

What is herewith presented is not to be considered in any sense a new method, but rather the description of a technic which has proved highly accurate and which can be recommended where small differences are to be detected, without resort to the laborious gravimetric method of Windaus and its modifications.

*Extraction*.—In Bloor's method and that of Sackett's modification, the drop precipitation in an alcohol ether mixture causes I believe a most complete extraction, and one even more complete than can be obtained by any method in the case of tissues. But the final heating of the precipitate to the boiling point causes a brownish pigment to disperse throughout the mixture. Whether as Luden contends, this interfering coloration is due to bile derivatives or not, I do not know, but it gives rise in the Burchard-Liebermann reaction to a confusing greenish shade which renders comparison with a standard solution sometimes inexact. Moreover, the amount of this brownish pigment seems proportional to the length of time the precipitate is heated. The difficulty can be overcome by omitting this heating altogether. Such extraction at room temperature is not at the expense of completeness, for the residue after filtration may be dried and extracted with chloroform in a Soxhlet for an hour without obtaining sufficient cholesterol to give even a faint Liebermann reaction.

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*Volumes and Temperatures of Solvents*—Larger volumes, more washings and close regard for constant temperature in measurements are all of importance. Three cc of blood are precipitated in 75 cc of a 2 to 1 alcohol-ether mixture and the flask, as well as the residue on the filter paper is washed with an additional 50 cc of the same mixture. This is reduced by distillation to about 90 cc and made up to 100 cc in a volumetric flask at a standard temperature (in this work  $21.5^{\circ}\text{C}$ ). Of this, 50 cc are at once pipetted into a 150 cc Erlenmeyer flask and evaporated almost to dryness in a boiling water-bath, the last few drops being removed in vacuo, while the flask is still hot, in order to avoid overheating of the residue, a notorious way of spoiling the color reaction. During the evaporation on the water-bath the flask should be topped by a small funnel to avoid loss by splashing. The dried residue and the funnel are then washed three times with 7 cc portions of boiling pure chloroform, these extracts united and made up to 25 cc at  $21.5^{\circ}\text{C}$ . From this flask 10 cc are taken for the colorimetric comparison.

*Standard Solution*—It is seldom stated by authors whether in making their standard solution they have used free cholesterol, an ester, or a mixture. Some commercial preparations merely labelled "Cholesterol" were found to be virtually all ester. Where comparative results alone are required either free cholesterol or an ester may be used as a standard when measuring absolute amounts the standard must be of known composition. The standard used here was made from pure free cholesterol. The latter was made from commercial preparations by hydrolysis with sodium ethylate and repeated recrystallization from alcohol and then from ether. Of this product, after thorough drying for twenty-four hours at  $100^{\circ}\text{C}$ , 50 mg were dissolved in 500 cc of pure chloroform at  $21.5^{\circ}\text{C}$ . The standard was kept in a glass stoppered bottle in the refrigerator to prevent evaporation and always slowly warmed to  $21.5^{\circ}\text{C}$  before taking a 10 cc sample for use.

*Colorimetric Determination*—There must be a separate standard specimen for each blood specimen, when several are being run through together, and both specimens treated in exactly the same way. Thus if there are three blood samples there will be three standard tubes, or six tubes in all. It matters a great deal whether the light employed is uniform or otherwise, whether the color reaction develops at the same or a different temperature in the two tubes, and whether both tubes do or do not mature for the same length of time.

I employed a single constant source of light, a 200 cp nitrogen bulb in a dark room. The bulb was installed on top of a desk and a circle drawn around it with red paint so that as many specimens as desired could be set equidistant from the light and thus receive equal illumination.

Temperature variations, even with a constant maturation time, give variations in coloration. Also, since the Liebermann reaction is exothermic, there often occurs rapid evaporation with blowing out of stoppers and consequent change in volume of the specimen. Temperature difficulties were reduced to a minimum in the following way. Glass-stoppered standard cylinders of 15 cc capacity were employed. Two cylinders, one containing 10 cc of the

chloroform extract and one containing 10 cc of the standard solution, were first of all immersed for two minutes in a bath of ice water which had previously been allowed to come to equilibrium in the refrigerator. This brought the temperature of the specimens to 3° C. Then 0.2 cc of pure H<sub>2</sub>SO<sub>4</sub> were added to each cylinder, the stoppers replaced, the cylinders shaken well and returned to the ice water bath for one minute to control the heat production. The cylinders were then removed and 2.0 cc of pure acetic anhydride added to each, both cylinders stoppered, inverted once to mix, and returned to the ice bath for a final one minute. Both were then removed and set upon the red line around the lamp. The time at which each pair of cylinders was first placed in the bath was taken as the zero hour from which the time for reading that pair was calculated. For all pairs, the same number of minutes elapsed between this first immersion and the reading. One pair can be thus prepared every five or six minutes and a reading can be made after maturation, in the same length of time.

With the temperature of the dark room at 18 to 20° C it was found that the cylinders thus prepared at a low temperature, develop their color slowly and at a regular rate. The low temperature usually causes a milky mess of the chloroform which always disappears on adding the anhydride. The cylinder containing the blood extract always matures a little in advance of the standard cylinder. Moreover the specimen cylinder usually passes through an early pink phase of color, a phenomenon not noted when the reaction occurs at higher temperatures.

After a period of forty five minutes from the time when the cylinders were first placed in the ice bath both sample and standard have reached maturity, a beautiful clear, bluish green color, which remains unchanged for a further period of fifteen minutes. It was my practice to make the comparison precisely at the end of forty five minutes.

A Duboseq colorimeter was used with the standard plunger usually set at 10 mm and ten successive readings made and averaged. It is well to have three or more strengths of standard solution on hand and to use the one which most nearly matches the color of the specimen.

#### Calculations

$$5 \times \frac{\text{standard reading}}{\text{specimen reading}} \times \frac{100}{3} = \text{number of milligrams per 100 cc of blood}$$

After a couple of weeks practice a surprisingly good degree of accuracy was developed by this technique as indicated by the following pairs of figures chosen at random, each pair representing parallel determinations on two 3 cc separate samples of the same specimens of whole blood of the dog

Specimen I	{ Sample 1 —126 mg per 100 cc
	{ Sample 2 —128 mg per 100 cc
Specimen II	{ Sample 1 —132 mg per 100 cc
	{ Sample 2 —130 mg per 100 cc
Specimen III	{ Sample 1 —138 mg per 100 cc
	{ Sample 2 —139 mg per 100 cc

In checking this method with the digitonin-precipitation gravimetric method, the technic of Fex<sup>6</sup> was used for extracting the 50 cc sample, the method of Gardner and Fox<sup>7</sup> was used for hydrolysis, and the method of Fraser and Gardner<sup>8</sup> for the estimation. The results of two such comparisons are given herewith:

I	}	Colorimetric estimation	175	mg	per 100 cc
		Gravimetric estimation	173.82	mg	per 100 cc
II	}	Colorimetric estimation	132	mg	per 100 cc
		Gravimetric estimation	149.36	mg	per 100 cc

The slightly higher figure obtained by the colorimetric method is not due, as is usually supposed, to interfering coloration (since there was none present) but is due, I believe, to the fact that alcohol-ether extraction is more complete than Fex's method can be made to be.

Throughout the present research on the behavior of blood cholesterol after meals, which will be separately published, I have allowed myself a 2 per cent error because this amount of error can be readily shown to be inherent in the actual reading of identical standard samples in the Duboseq instrument.

#### TISSUE CHOLESTEROL

The use of colorimetric methods for tissues has to some extent fallen into disrepute because of the frequency of interfering colorations. Where the latter can be avoided, as in the present work, there seems no objection to using the method. The whole problem here is to obtain a method of extraction at once convenient, thorough, and free from the major difficulty just referred to.

For two months I experimented with different methods of drying and the use of various solvents (chloroform, alcohol, ether, all at various temperatures) both directly and in Soxhlets, and in modified Soxhlets where the repeating extractions occurred at many temperatures from 10° C up.

All drying of tissues preparatory to extraction was finally abandoned. Similarly, all solvents were abandoned except ether, because the others took from the tissues substances responsible for interfering colorations in the Liebermann reaction.

Fex's method of extracting an alkaline emulsion of fresh tissue with ether, which enjoys the recommendation of Gardner and Fox<sup>7</sup> for gravimetric work, was found to satisfy the requirements well.

The required weight of minced fresh tissue is mixed with twice its weight or more of 2 per cent caustic soda and allowed to digest about twelve hours at room temperature. The flask is then put on a boiling water-bath for thirty or forty minutes. On removing and shaking, the tissue will be found to have gone almost completely into solution. (Where esters are to be estimated it is better to allow digestion to continue for only three or four hours.)

Fex then extracted in a separator with ether, permitting the mixture to stand twelve hours for complete separation. Gardner and Fox found it usually necessary to make more than two separations. I found this process could be greatly shortened and made slightly more complete by the following technic:

The cooled, alkaline digest is mixed by shaking with ether in a separator and a safe separation made at the end of eight or ten minutes. This process is repeated five times and the ether saved. Then three further extractions are done in glass stoppered centrifuge tubes the tubes being centrifuged at moderate speed for ten minutes each time to assist separation, and the ether siphoned off. Finally three more similar extractions are made in these centrifuge tubes, but with excess of  $\text{NaCl}$  added to the alkaline digest to hasten separation.

The ether extracts are united and washed three times with water to remove alkali, salt and other water soluble substances. This is facilitated by keeping the volume of the water small in comparison with the amount of ether at each mixing. The wash waters are then united and washed three times with ether, the ether washings being added to the ether extract. This process is facilitated by employing large proportionate volumes of ether.

This procedure was found to remove all but a trace of the cholesterol of the tissue, a trace too small to be estimated, but constituting possibly 0.05 per cent of the total amount of sterol present.

This washed ether extract is the same as employed by Gardner and Fox for gravimetric estimation. The author found that on distilling off the ether to dryness and on taking up the residue in hot chloroform, and then proceeding as in the technique for blood, a perfect color match was obtained for comparison with a standard cholesterol solution.

The same character of standard solution, and the same precautions as to light, temperature, and maturation time were employed as described in the first section of this article.

### Calculation

$$\frac{\text{Standard reading}}{\text{Sample reading}} \times \text{dilution of sample} = \text{number of milligrams in the tissue extracted}$$

Occasionally, but very seldom a slight discoloration may occur in the sample cylinder being indicated by a brown color on the addition of sulphuric acid and a muddy green on the addition of the anhydride. When this occurs it is due either to insufficient washing with water, or to imperfect separation in the separator.

One should be prepared also for the occurrence of unusual but harmless pinks salmon shades and purples which sometimes develop early in the cold sample cylinder and which represent phases of color development preliminary to the final bluish green seen at the end of forty five minutes.

In checking this technique against the gravimetric, the modification of Fex's method was used for extraction, the method of Gardner and Fox was used for hydrolysis and that of Fraser and Gardner for estimation.

In order to obtain a sufficiently large amount of cholesterol for ease in handling, some commercial cholesterol was added to a Fex extract of cow's gastric mucosa (tripe). Aliquot portions of this solution were taken and subjected to both gravimetric and colorimetric estimation.

# Results

Sample I	{ By colorimetric method,	0 199 grams
	{ By gravimetric method,	0 196 grams
Sample II	{ By colorimetric method,	0 216 grams
	{ By gravimetric method,	0 215 grams
Sample III	{ By colorimetric method,	0 202 grams
	{ By gravimetric method,	0 206 grams

I confess that I found the gravimetric technique extremely difficult and that, in my hands, it contained a 2 per cent maximum error which was no better than the 2 per cent error in the colorimetric. But, for the comparative purposes of the problem for which the technique was developed, it seemed to satisfy the requirements quite well.

*Control Used to Correct for Blood Contained in Tissue*—Where one wishes to know the amount of cholesterol in a piece of tissue it is desirable to make a deduction for blood contained in the tissue, the balance representing the actual tissue sterol proper. This can be readily done if, in doing periodical tissue sampling, a blood sample be taken from a vein at approximately the same time. The tissue on removal is washed with water, blotted, weighed and minced. The minced tissue is placed in a 0.4 per cent solution of ammonia and set on the desk for two hours with occasional shaking. With this treatment the tissue pales and the hemoglobin of the contained blood passes into solution imparting a pink or red color to the ammonia solution. Some of this solution is then poured into a graduated cylinder, and into an identical cylinder containing an equal volume of fresh 0.4 per cent ammonia, some venous blood is dropped from a calibrated pipette until the colors match.

## Calculation

$$\frac{V \times T \times C}{t} = \text{amount of blood cholesterol in tissue, where}$$

V = amount of venous blood in c.c.

T = volume of ammonia into which tissue was placed

C = amount of cholesterol in 10 c.c. of venous blood sample

t = volume of ammonia from tissue flask used for the hemoglobin comparison

When the comparison is finished, the ammonia solution removed from tissue flask is poured back into the flask, and enough caustic soda added to render the solution 2 per cent, as required in Fessenden's method. During the subsequent heating on the water-bath, the ammonia is of course driven off.

Appreciation and cordial thanks are tendered to Dr. V. E. Henderson and Dr. G. H. W. Lucas for much consultation and assistance in theory and technique.

## REFERENCES

- Bloor, W. R., and Knudson, A. Cholesterol and Cholesterol Ester, Separate Determination in Small Amounts of Blood, *Jour Biol Chem*, 1916, **xxvii**, 463.
- Bloor, W. R. Determination of Cholesterol in Blood, *Jour Biol Chem*, 1917, **xxix**, 437.
- Sackett, G. E. Modification of Bloor's Method for Determination of Cholesterol in Whole Blood or Blood Serum, *Jour Biol Chem*, 1925, **lxiv**, 203-205.
- Myers, V. C., and Wardell, E. L. Colorimetric Estimation of Cholesterol in Blood, etc., *Jour Biol Chem*, 1918, **xxvi**, 147.
- Leiboff, S. L. Simplified Method for Cholesterol Determinations in Blood, *Jour Biol Chem*, 1924, **lxi**, 177-180, also *JOUR LAB AND CLIN MED*, 1925, **v**, 857-61.

- Luden, G Influence of Bile Derivatives in Blood Cholesterol Determinations, *JOUE. LAB AND CLIN. MED* 1917, iii 93 also *Jour Biol Chem*, 1917, xxix, 463
- \*Fox, J Chemische und morphologische Studien über das Cholesterin und die Cholesterinester in normalen und pathologische veränderten Organen, *Biochem Ztschr* 1920, civ, 82-174
- Gardner, J A, and Fox, F W A Critical Study of the Methods of Estimating Cholesterol and its Esters in Tissues Part II *Biochem Jour* 1924, xviii, 1058-69
- \*Fraser M I, and Gardner, J A Estimation of Free Cholesterol *Proc Roy Soc Lond, Series B* lxxxi p 559-67

## CELLULOID CORROSION TECHNIC FOR STUDY OF NORMAL AND PATHOLOGIC VARIATIONS OF THE ARTERIES OF THE KIDNEY\*

By NELSON W BARKER M D ROCHESTER MINNESOTA

THE celluloid corrosion method for demonstrating and studying blood vessels has been used widely for many years. Schiefferdecker, in 1882 was the first to describe the method and since then numerous articles in the literature describe improvements in the composition of the injection mass, in the types of apparatus and in the method of carrying on the injection.

The technic used at The Mayo Clinic in a study of the normal and pathologic variations of the arteries of the kidney is fundamentally that reported by Huber in 1906, with the modifications by Hinman, Morrison and Lee Brown in 1923, by Morrison in 1926, by McIndoe, and by Counseller and McIndoe, in 1926, in their work on the liver. It is adaptable for different organs, but slight changes are necessary for each. It is possible to inject the arterial system of the normal kidney completely and consistently as far as the capillaries, and the method has been used to compare the arterial casts of normal kidneys with those exhibiting various types of renal lesions particularly nephritis.

### TECHNIC

*Removal of the Organ*—In the removal of the organ the intact kidney, the intact renal artery with a small portion of the aorta attached and a small amount of perirenal connective tissue without a great deal of perirenal fat are included. The tissues should be obtained as soon as possible after death and should not have been previously subjected to formalin embalming, or preserving solutions. The reason for this is that technical difficulties are in direct proportion to the degree of postmortem degeneration which has taken place. Usually if more than five hours elapse after death before the specimen is obtained the walls of the vessels may be considerably softened and weakened.

*Cannulas*—Glass cannulas with smooth tips and definite but smooth shoulders are used. The cannula is inserted into the aortic orifice of the

Work done in the Section on Pathologic Anatomy, The Mayo Clinic. Read before the International Association of Medical Museums, Washington, D C, April 30, 1928.  
Received for publication May 26, 1928.



renal artery and tied tightly behind the shoulder with a strong string or heavy thread

*Washing*—The cannula is then connected with the cold-water tap by a rubber tube and the tap turned on slightly. If the pressure becomes too great the tube will separate from the cannula. Washing is carried on with the kidney immersed in cold water to prevent, as far as possible, further post-mortem degeneration. The washing is kept up for three hours or longer if the water coming from the renal vein is still blood-stained. Warm physiologic sodium chloride solution has not been found to possess any advantage over cold tap-water for washing.

*Drying*—The purpose of the drying is to remove only a moderate amount of water from the kidney and collapse the larger vessels. The kidney is wrapped in towels and placed in an ice box under a 500 gram weight for at least two hours. It may be left there as long as twelve hours.

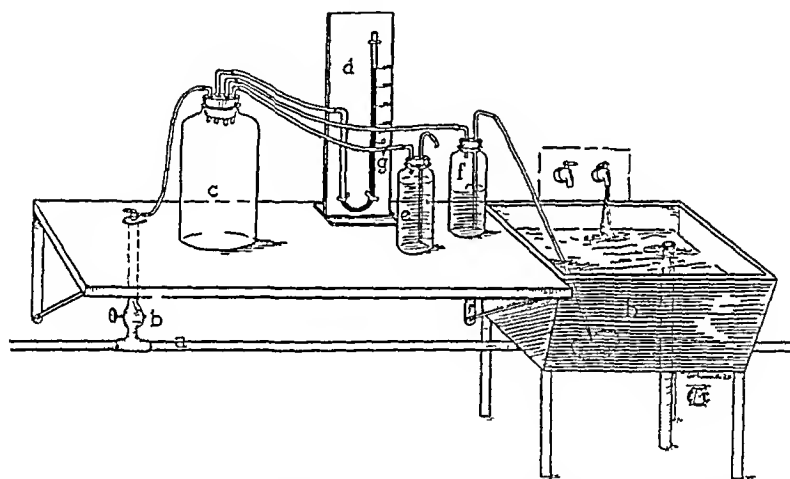


Fig 1—Injection apparatus. *a*, air line. *b*, needle valve. *c*, pressure bottle. *d*, mercury monometer. *e* and *f*, injection bottles. *g*, clamp. *h*, sink with running water.

*Leaks*—To prevent leaks all the small branches of the main renal trunk are tied as well as all the perforating capsular arteries which were necessarily torn or cut in removing the kidney from the body. This procedure is very important since leaking constitutes the major technical difficulty. An uncontrollable leak will spoil the whole injection. The method I have used is to hold the kidney under water and either blow or force air under slight pressure through a tube attached to the cannula. The small torn vessels are identified by the bubbles, caught with artery forceps and tied carefully and thoroughly with a heavy thread.

*Apparatus*—Fig 1 shows the apparatus used (modified from that used by Counseller and McIndoe). Formerly a carbon dioxide tank was used as a source of pressure. An air line with continuous pressure as high as 500 mm of mercury from a machine pump is preferable to a gas tank and is used now for all such work at The Mayo Clinic. The needle valve is important since it regulates the pressure for the entire apparatus. The pressure bottle has a sealed cork with four glass tubes perforating it. One is attached to

the air line, one to a mercury manometer and two are available for injection bottles. By attaching Y tubes to these the number of injections carried on at a certain pressure can be increased indefinitely. The injection bottles hold 600 c c. Rubber corks are perforated by a long and a short glass tube and are tied in tightly when the bottles are filled with the injection mass. All glass tube ends are flanged and the rubber connections tied tightly behind these flanges.

*Injection mass*—Old roentgen ray films are a cheap and satisfactory source of colloidin. The silver emulsion is washed off and the films thoroughly dried. As a stock solution the mass is made up as follows: roentgen ray films, 10 gm., gum camphor 8 gm., acetone, 100 c c. For deep injection, one part of this mass is diluted with four parts of acetone, making a formula: roentgen ray films, 2 gm., gum camphor, 16 gm., acetone 100 c c. For coloring matter alkanet (first described by Huber) has been used, the dye being extracted from crude alkanet root with acetone. It is not an attractive red but it is soluble in acetone and unchanged by strong hydrochloric acid. If contrast colors are desired, Windsor and Newton oil paints (described by Counseller and McIndoe) are satisfactory, but they are not soluble in acetone and the suspension does not penetrate as deeply as the mass with a soluble dye.

*Injection*—The apparatus is connected and the tube leading from the long arm of the injection bottle is filled with the injection mass and clamped near its end. This is connected with the cannula in the renal artery and tied tightly over it. The pressure is then raised to 400 mm. of mercury and the clamp released, allowing the mass to enter the renal artery suddenly at this pressure. Probably the maximal penetration is secured during the first few minutes. If there are any other small leaks they are immediately controlled by clamping and tying. At the end of an hour the pressure is reduced to 200 mm. of mercury, and kept there for from seven to ten days. The purpose of this is to insure solid branches. Thicker solutions of the mass may be added to the original injection bottle one or two days after the beginning of the injection if it is desirable to shorten the time of the injection. Care is taken not to permit air to enter the injection tube. During the injection the kidney is immersed in a sink filled with cold running water. At the end of the injection a clamp is placed on the tube near the cannula and the tube cut above it. The kidney is then allowed to remain in the water for twenty-four hours.

*Corrosion*—The cannula is cut out of the end of the artery and the kidney placed in commercial hydrochloric acid. After from two to four days it is taken out and washed and if corrosion is not complete it is replaced in the acid and the process repeated until the cast is clean. The kidney is washed by immersing it in water and playing a fine stream of water on it, the washing cannula is also held under water. Care is taken not to lose any small branches of the cast.

*Mounting*—About a fourth of the cast is cut away in order to expose the internal branches and to have material for microscopic examination. This portion is kept in water and the remainder of the cast is dried, then sprayed with a thin solution of clear varnish from an ordinary throat spray and mounted.

dry on a sheet of heavy white celluloid in a partial vacuum jar, according to the method of Lundquist and Robertson. Small fragments are cut from the piece kept in water and placed on hanging-drop slides in water to be studied with the microscope or they may be permanently mounted on these slides in glycerin and the cover slips sealed with asphalt. They can be studied best with a 32 mm objective.

*Comment*—The important points in the technique are: (1) complete washing of the vascular system of the specimen, (2) tying off all main vessels, (3) a thin injection mass: roentgen-ray films 2 gm, camphor, 16 gm, acetone, 100 cc, (4) high initial pressure, 400 mm of mercury, (5) maintenance of the injection under pressure of 200 mm of mercury for from seven to ten days, and (6) careful washing of the cast.

#### ADVANTAGES OF THE METHOD

- 1 The casts are easily handled, studied, demonstrated, photographed and preserved, or they may be cut up, and the course of each vessel traced.
- 2 The arterial tree only is injected and this as far as the capillaries. The veins are not injected and thus do not obscure the picture.
- 3 Small fragments of the cast may be studied under the binocular microscope, thus details of the finer arterial branches are revealed.
- 4 The presence or absence of anastomosis is easily detected.

#### DISADVANTAGES OF THE METHOD

- 1 The destruction of the kidney precludes the possibility of obtaining microscopic sections. However, if the two kidneys are of approximately equal size and weight, and gross abnormalities are not present, microscopic sections from one may be taken as a fairly good index of the other. Furthermore, if it is necessary to take a section from a kidney to be used for celluloid injection, it can be taken from one pole and the main interlobar branch of the renal artery to this region ligated. This can usually be identified before it enters the hilum. The end artery distribution in the kidney makes it possible to do this and still inject celluloid into the remainder of the kidney without leaks.
- 2 The casts are fairly fragile.
- 3 The casts are only of the lumina of the vessels at their maximal dilatation. The arterial walls have corroded away and cannot be studied.

#### REFERENCES

- <sup>1</sup>Counseller, V S, and McIndoe, A H. Dilatation of the Bile Ducts (Hydrohepatosis), Surg, Gynec, and Obst, 1926, *liii*, 729-740.
- <sup>2</sup>Hinman, Frank, Morrison, D M, and Lee Brown, R K. Methods of Demonstrating the Circulation in General, Jour Am Med Assn, 1923, *lxvi*, 177-184.
- <sup>3</sup>Huber, G C. The Arteriolae Rectae of the Mammalian Kidney, Am Jour Anat, 1906, *vi*, 391-406.
- <sup>4</sup>Lundquist, R L, and Robertson, H E. The Dry Mounting Method for Museum Specimens, Hosp Prog, 1926, *vii*, 273-275.
- <sup>5</sup>McIndoe, A H. Personal Communication.
- <sup>6</sup>Morrison, D M. A Study of the Renal Circulation With Special Reference to Its Finer Distribution, Am Jour Anat, 1926, *xxxvii*, 53-71.
- <sup>7</sup>Schnefferdecker, P. Ueber die Verwendung des Cellodins in der anatomischen Technik, Arch f Anat u Entwicklungsgesch, 1882, 199-203.

## TUBERCLE BACILLI AND ELASTIC TISSUE\*

### A RAPID METHOD FOR STAINING BOTH IN THE SAME PREPARATION

BY ISRAEL RAPPAPORT M D AND RICHARD T. LEVISON B S M D PHILADELPHIA

EVER since Ballou<sup>1</sup> pointed out the great diagnostic and prognostic significance of elastic tissue in the sputum of cases of pulmonary tuberculosis the need for a method rapid enough for routine clinical use has been felt. Many methods of elastic tissue staining have been worked out and in many sanatoria for tuberculosis one or another of such methods has become part of the routine sputum examination. However, mass observation of the types and occurrence of elastic tissue in the various clinical forms of pulmonary tuberculosis has not appeared chiefly because the methods were tedious and time consuming and necessitated the preparation and staining of a second smear in addition to the one stained for tubercle bacilli. Many attempts have been made to develop a simple method of staining both tubercle bacilli and elastic tissue in the same preparation the most successful of these in recent years being those of Bezancon and Brodiez in 1922 and Jessen<sup>2</sup> in 1926. These methods permit the staining of tubercle bacilli and elastic tissue in the same preparation but are excluded from the routine of the busy clinical laboratory because of the time required thirty minutes at the least.

In our endeavor to investigate the occurrence and type of elastic tissue appearing in the sputum of patients with pulmonary tuberculosis we have worked out a modification of Weigert's elastic tissue stain. This permits of a rapid staining of the elastic tissue in a simple direct smear. We found that the tubercle bacilli also take this stain in the same time and that by gentle decolorization and counterstaining we had a smear in which all the tubercle bacilli as well as the elastic tissue fibers remained stained by the basic fuchsin. The method is really so very simple and quiet that it proved to be more efficient as the routine method for staining tubercle bacilli even in cases in which elastic tissue was not to be looked for than the usual Ziehl-Neelsen technique. After careful comparison we have convinced ourselves that it very often shows tubercle bacilli when no other method will do so. The reason for this appears to us to be in the fact that this method entails considerably less tampering with the organisms than any of the other methods; no strong acids are used, no heating is done and only three steps taking in all only five minutes are necessary.

#### SOLUTIONS AND METHOD

##### *Solution 1*

Basic fuchsin	2 gm
Resorcin	4 gm
Water	100 cc

From the Home for Consumptive, Chestnut Hill, Phila.  
Received for publication May 1 1926

Dissolve and boil three minutes and then add

Liquor ferrisessquichloride

15 cc

Boil three minutes then filter and allow sediment to dry on filter paper for about one half hour

Dissolve sediment on filter paper in

Alcohol 95 per cent

100 cc

Boil for a few minutes and filter Bring volume of filtrate to 100 cc with additional 95 per cent alcohol and add

Hydrochloric acid c p

3 cc

Note Any basic dye such as orcein, safranine, etc., can be substituted for the fuchsin

#### *Solution 2*

Hydrochloric acid c p

3 cc

Alcohol 95 per cent

100 cc

#### *Solution 3*

Aqueous methylene blue

A selected particle of sputum should be smeared by gentle stroking in such a way that layers of varying thickness are produced, because bacilli are more easily seen in thin layers and elastic fibers are more apt to be found in thick layers Too great maceration should be avoided as this may break up the natural relation of bacilli and elastic tissue

1 The dried smear is flooded with the stain (Solution 1) from three to five minutes according to the strength of the stain

2 The rinsed smear is then decolorized (Solution 2) to a faint pink and rinsed again

3 Counterstain with aqueous methylene blue

By this method both bacilli and elastic fibers appear a bluish purple, the fibers usually more deeply stained than the bacilli The bacilli appear swollen and unusually large in spite of the fact that only the interior takes the dye and the waxy capsule appears as a highly refractile shell inclosing the purplish rod Occasionally this waxy capsule may contain very little or none of the dye These we have considered as being bacilli possessing a minimum of acid fastness that would have prevented their recognition by a purely acid fast staining method The elastic fibers stain various shades of bluish purple depending on their thickness There are no other objects appearing in the sputum that can be confused with these fibers

We have been using this method for the past two years and we have found that the routine report as to the presence or absence of elastic tissue has been of material help in the general consideration of a case both from the standpoint of prognosis as well as progress Following the lead of Bezancon and Biodiez we have divided the elastic tissue fibers into classes according to their general appearance We found it better, however, to restrict our classification to the two main types commonly recognizable E T 1 are fine distinct fibers that retain wholly or in part their alveolar shape After seeing a few examples this type is unmistakable E T 2 occurs in bundles often frayed at the ends or in single heavy strands We

found that in pulmonary tuberculosis the finding of E T 1 is definitely pathognomonic of widely disseminated rapidly softening process. With one exception patients repeatedly showing this type of elastic tissue have progressed through a very active clinical course to a fatal termination. E T 2 is the usual type seen in pulmonary tuberculosis and occurs in all other forms of this disease.

Elastic tissue fibers can be demonstrated at some period in the course of every open case of pulmonary tuberculosis. They are rarely if ever found in the sputum of tuberculous patients if bacilli are absent, although concentration methods or animal inoculation may be necessary to demonstrate the bacilli. The usual story is first the appearance in the sputum of tubercle bacilli followed in a short time by both bacilli and elastic tissue. This condition persists during the active course of the disease to be followed as quiescence or recovery occurs by the disappearance first of the elastic tissue and then of the bacilli.

#### REFERENCES

- \*Ballin Berl klin Wchnschr 1920, xxx, 733  
 \*Bezancon and Brodick Rev d I Tuberc 1922, ix 398

### A NEW BLOOD TAKING TUBE\*

By AUGUST STEPHEN WOLF, NEW YORK

ALTHOUGH there are at the present time various forms of apparatus being used for obtaining blood from a vein, the one which I am about to describe may from my experience be of interest to others (Fig 1)

Fig 3 A, container (capacity of 50 cubic centimeters), B, tubulation, C, ground nib, D, perforated stopper, E, trap containing cotton, F, rubber tubing, G, mouthpiece H, Luer needle

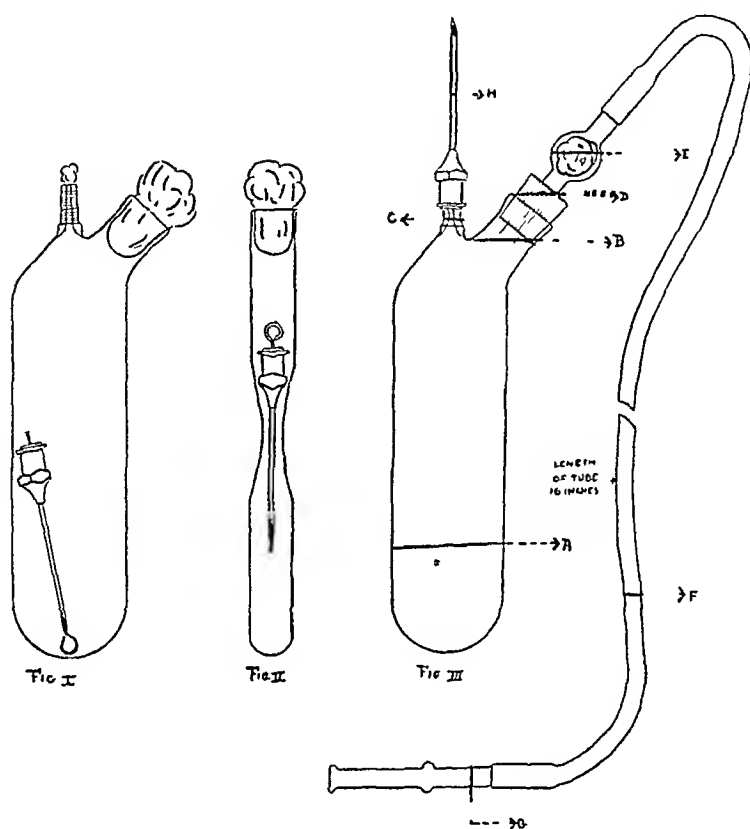
For the purpose of elucidation in the use of this tube I shall make mention of a part of the technique for obtaining blood. To insure the positive exclusion of air it is advisable to smear a little vaseline around C. Connect D, E, F, and G. Insert D in B and connect H and C. Place the subject in a reclining position, flat on the back,† with the arm from which the blood is to be taken in a suspended position. Select a prominent vein, sterilize the site, and apply the tourniquet. Place G in the mouth, produce suction, and insert the sterilized needle, taking precaution to hold B upward (for if it is not in this position, the blood will gravitate into the tubulation and thereby be drawn into the mouth of the operator).

From the Laboratory of Equitable Life Assurance Society, New York.

†This prevents to a great extent cerebral anemia.

The above mentioned outfit can be obtained from The Empire Laboratory Supply Company, Incorporated 549 W 1<sup>st</sup> St New York New York.

It might not be amiss to draw the attention of the reader to some of the advantages of the outfit mentioned (1) Any size needle can be used, (2) it can be sterilized in the tube proper (see Fig 1) or in a separate receptacle (see Fig 2), (3) the tube can be cleansed and used over again, (4) it can be used for bacteriologic as well as chemical serologic, and other blood investi-



WOLF BLOOD TAKING TUBE

gations, (5) it is made to fit a 50 c c centrifuge shield, (6) parts can be replaced independently, (7) it is necessary to use but one hand, as the tube functions as a handle, (8) blood is visible as soon as the needle pierces the wall of the blood vessel, (9) the specimen is in such form as to allow of pouring from the tube into other receptacles and adding such chemicals as are necessary

## A HEAD CLAMP FOR DECEREBRATE ANIMALS\*

J. A. INOCH KAPRER PH.D. AND H. C. STLAENS PH.D., M.D. CLEVELAND

IN THE course of some recent work on the measurement of muscular tension in decerebrate cats it was found convenient to mount the preparation on a canvas frame in which there were openings through which the legs were thrust. To hold the head upright in order to facilitate respiration, it was found advantageous to construct the head clamp which is here pictured in Fig. 1 and described. Two bars are hinged together the one A being straight and having riveted on its end a small piece of wood B or rubber from the tread of an automobile tire the other bar C is curved along its length in

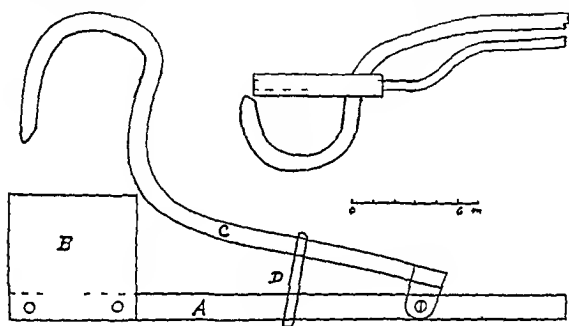


FIG. 1

several directions and is hooked on its end to fit the shape of the neck under the occipital protuberance. When ready to use the head clamp, the bars are opened like a pair of shears and the wood (or rubber) piece is inserted between the teeth and the hook is placed about the neck.

The two bars are held together by the band, D, which by being forced along the bars locks them together. The end of the head clamp is fastened to a vertical rod by means of an ordinary laboratory clamp. The grip of the head clamp is so secure that a decerebrate animal could be freely suspended by the head, if necessary, without mechanical interference with respiration or circulation. A head holder of this type could be usefully applied to the human cadaver for the purpose of holding the head rigid while sawing through the skull, in the performance of a postmortem examination.

\*From the H. K. Cushing Laboratory of Experimental Medicine Western Reserve University, Cleveland, Ohio.

Received for publication April 3, 1918.



## A SPECIAL KNIFE FOR DECEREBRATION\*

BY ENOCH KARRER, PH D, AND H C STEVENS, PH D, M D, CLEVELAND

THE instrument here described is designed for the decerebration of cats and animals of like size through a trephine opening in the skull. It is especially designed to produce the particular type of decerebration known as the thalamus preparation. The instrument is drawn in Fig 1, in which 1 is a

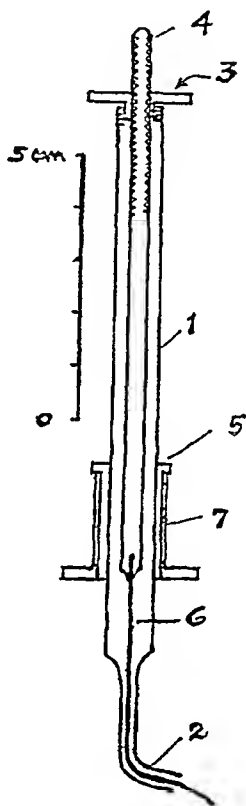


Fig 1

small steel tube having at its one end a flat and smaller portion 2, with relatively sharp edges, and having on its other end a rotatable but not translatable nut, 3. Through the nut 3, passes a portion of a screw 4, extending down into the tube 1, and terminating in a flat portion of steel, or other material, 6 whose edges may be sharpened to enable it to cut through the tissues inside the cranium. This flattened portion is also slightly curved downward. The whole tube 1 may be rotated in a sleeve 7 where tube 5 affords a bearing that

\*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

Received for publication April 23, 1928.

may be slid along tube 1. The extensible knife edge 6 is withdrawn by manipulation of screw 4 and the end 2 is inserted into the cranial cavity through an opening in the skull situated in the median plane approximately 1 cm caudad to a straight line joining the outer angles of the posterior borders of the two orbits of the eyes. The depth of introduction is determined by the position of the sleeves 5 and 7. In case of average size cats this distance should be from 1 to 1.6 mm. The extensible knife edge 6 is then caused to project by turning the nut 3 until the end of it is felt to be against the cranial wall. Then the whole tube is rotated in 7 to cut the tissues that will fall on the surface described by the cutting edge. The knife when manipulated in accordance with the directions given above passes through the brain just cephalad to the optic thalamus. The projecting cutting edge, 7, is best made of rustless steel or other material as sheet molybdenum. When made of high carbon steel, rusting is serious through the narrow tube 2.

## A PYREX GLASS BURNER FOR THE PRODUCTION OF SODIUM LIGHT\*

By EDWARD S. WEST, PH.D. ST. LOUIS

CALDWELL and Whymper<sup>1</sup> obtained an intense sodium light by passing finely powdered sodium carbonate into a blowpipe flame along with the air supply. The constancy of the flame varied considerably. These workers also bubbled gas through a mixture of sodium carbonate and sand contained in a bottle, in the neck of which a metal Meeker burner tube was mounted, and obtained a steadier though less intense flame. This latter principle of Caldwell and Whymper was adapted to an apparatus of pyrex glass. Because it has proved especially satisfactory as a source of light for polariscopic work a description of its construction is given.

The apparatus consists essentially of two parts, first a chamber for fine sand and salt through which the gas supply bubbles, and second a burner of the Bunsen type placed above it. The gas bubbling through the sand salt bed, carries particles of salt in suspension through the burner and produces a brilliant sodium flame when ignited at the tip. The salt sand chamber consists of a pyrex tube approximately 25 by 160 mm fitted at the bottom with a gas inlet tube and near the middle with a wide side tube which serves as a salt reservoir. The chamber is preferably somewhat enlarged at the bottom as shown in the photograph, though this is not essential. The gas inlet tube is passed through the wall of the salt sand chamber near the bottom until it almost touches the opposite side. It is closed at the end and provided with a small aperture (about 1 mm) on the under side and in the center of the chamber. By this arrangement the gas passes first down into the salt mixture and then up through it. A small opening for the gas inlet into the chamber is

<sup>1</sup> Demonstrated in preliminary form at the meeting of the American Society of Biological Chemists Cleveland Ohio Dec 8 to 31 1915

From the Department of Biological Chemistry Washington University School of Medicine.

desirable to insure sufficient bubbling pressure and an even flow of gas. The layer of sand should extend a few millimeters above the gas inlet tube, and the quantity of salt should be such that the gas readily passes through it. The supply of salt in the chamber is replenished as needed by tapping the walls of the salt reservoir. A larger salt-sand chamber and reservoir may be constructed if greater capacity is desired. The burner is conveniently made from heavy pyrex tubing of about 10 or 12 mm bore. The air inlet tubes of 7 or 8 mm bore are located about 45 mm from the end of the main burner tube and curved down. They should be a few cm in length. The burner

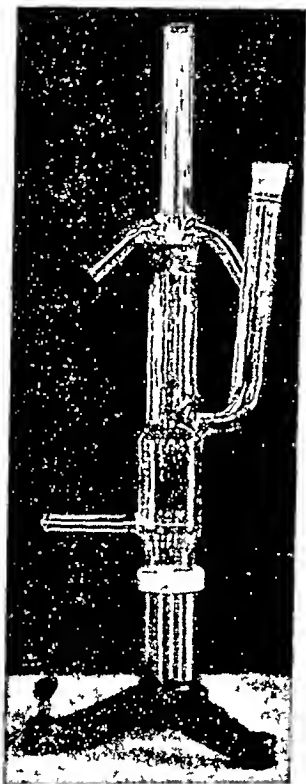


Fig 1

should be long enough to give an evenly burning flame without flicker, about 150 or 160 mm. The inner tube through which gas passes from the salt-sand chamber into the burner may be either sealed in or more simply secured with a rubber stopper. *A very essential point is to adjust the gas and air intakes of the burner so that a hot, blue roaring flame is obtained.* This is easily done by sealing on the air inlet tubes of such size that an excess of air is assured, and then attaching a rubber tube with screw clamp to one of the inlet tubes. By opening and closing the clamp the air supply may be regulated at will. The burner is attached to the salt-sand chamber with a rubber stopper. The specifications given may be changed within rather wide limits with satisfactory results. The salt reservoir tube should be sloped so that salt runs into the chamber only upon tapping.

may be slid along tube 1. The extensible knife edge 6 is withdrawn by manipulation of screw 4 and the end 2 is inserted into the cranial cavity through an opening in the skull situated in the median plane approximately 1 cm caudad to a straight line joining the outer angles of the posterior borders of the two orbits of the eyes. The depth of introduction is determined by the position of the sleeves 5 and 7. In case of average size rats this distance should be from 1 to 1.6 mm. The extensible knife edge 6 is then caused to project by turning the nut 3 until the end of it is felt to be against the cranial wall. Then the whole tube is rotated in 7 to cut the tissues that will fall on the surface described by the cutting edge. The knife when manipulated in accordance with the directions given above passes through the brain just cephalad to the optic thalamus. The projecting cutting edge, 7, is best made of rustless steel or other material as sheet molybdenum. When made of high carbon steel rusting is serious through the narrow tube 2.

## A PYREX GLASS BURNER FOR THE PRODUCTION OF SODIUM LIGHT\*

By EDWARD S. WEST PH.D. ST. LOUIS

CALDWELL and Whimper<sup>1</sup> obtained an intense sodium light by passing finely powdered sodium carbonate into a blowpipe flame along with the air supply. The constancy of the flame varied considerably. These workers also bubbled gas through a mixture of sodium carbonate and sand contained in a bottle, in the cork of which a metal Meeker burner tube was mounted, and obtained a steadier though less intense flame. This latter principle of Caldwell and Whimper was adapted to an apparatus of pyrex glass. Because it has proved especially satisfactory as a source of light for polariscopic work a description of its construction is given.

The apparatus consists essentially of two parts first a chamber for fine sand and salt through which the gas supply bubbles, and second a burner of the Bunsen type placed above it. The gas bubbling through the sand salt bed, carries particles of salt in suspension through the burner and produces a brilliant sodium flame when ignited at the tip. The salt sand chamber consists of a pyrex tube, approximately 25 by 160 mm fitted at the bottom with a gas inlet tube and near the middle with a wide side tube which serves as a salt reservoir. The chamber is preferably somewhat enlarged at the bottom as shown in the photograph though this is not essential. The gas inlet tube is passed through the wall of the salt sand chamber near the bottom until it almost touches the opposite side. It is closed at the end and provided with a small aperture (about 1 mm) on the under side and in the center of the chamber. By this arrangement the gas passes first down into the salt mixture and then up through it. A small opening for the gas inlet into the chamber is

<sup>1</sup> Demonstrated in preliminary form at the meeting of the American Society of Biologists Chemists Cleveland Ohio Dec. 8 to 31 1915

From the Department of Biological Chemistry Washington University

desirable to insure sufficient bubbling pressure and an even flow of gas. The layer of sand should extend a few millimeters above the gas inlet tube, and the quantity of salt should be such that the gas readily passes through it. The supply of salt in the chamber is replenished as needed by tapping the walls of the salt reservoir. A larger salt-sand chamber and reservoir may be constructed if greater capacity is desired. The burner is conveniently made from heavy pyrex tubing of about 10 or 12 mm bore. The air inlet tubes of 7 or 8 mm bore are located about 45 mm from the end of the main burner tube and curved down. They should be a few cm in length. The burner

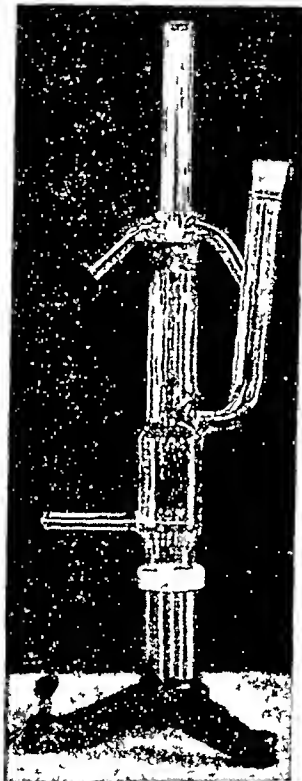


Fig 1

should be long enough to give an evenly burning flame without flicker, about 150 or 160 mm. The inner tube through which gas passes from the salt-sand chamber into the burner may be either sealed in or more simply secured with a rubber stopper. *A very essential point is to adjust the gas and air intakes of the burner so that a hot, blue roaring flame is obtained.* This is easily done by sealing on the air inlet tubes of such size that an excess of air is assured, and then attaching a rubber tube with screw clamp to one of the inlet tubes. By opening and closing the clamp the air supply may be regulated at will. The burner is attached to the salt-sand chamber with a rubber stopper. The specifications given may be changed within rather wide limits with satisfactory results. The salt reservoir tube should be sloped so that salt runs into the chamber only upon tapping.

With one charge of sand and salt (including the salt reservoir), a relatively constant light of high intensity can be obtained for one or more hours depending upon the rate of gas flow, fineness of salt, etc. Satisfactory salt can be prepared by very finely grinding a *re p* grade of sodium chloride or good free running table salt in a mortar or preferably in a ball mill and then drying by heating in a metal dish with a free flame. The sand used should be rather fine and of round grain to insure good bubbling. It also should be dried by heating. The gas supply should be passed through a drying tower of calcium chloride before entering the apparatus, otherwise the salt and sand will cake.

This type of sodium burner has been found more satisfactory for both student and research use than any other I have used. White and Rabinowitch found it to be satisfactory as a source of light in measuring the optical rotation of sugar solutions which had been placed in Thurn Vella loops of dogs.

#### REFERENCES

- <sup>1</sup>Caldwell and Whymper Proc Roy Soc, (A) lxxxi 112  
<sup>2</sup>White, H. L., and Rabinowitch J Jour Biol Chem 1927 lxxiv 449

#### Errata

In the article by Greenbaum entitled 'New Iodo Derivatives of Phthaleins,' October, 1928, issue, on page 40, first line should read "The introduction of iodine into a nitrated phenolphthalein resulted in a etc."

On page 41, fifth line, 18° C should be 180° C

On page 42 first line of third paragraph, word 'detoriodophenolphthalein' should be "octoiodophenolphthalein"

On page 45 line seven word 'metacresolphthalein' should be 'ortho cresolphthalein'

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## LABORATORY TECHNIC

**VAN DEN BERGH TEST** A Study of the Properties of Pure Bilirubin and Its Behavior Toward the Van Den Bergh Reagent, Davies, D. T., and Dodds, E. C. *Brit Jour Exper Path*, 1927, viii, No. 4, p. 316

The authors' experiments suggest the following

That the behavior of bilirubin toward the diazo reagent is constant, and that changes are either due to alteration in the  $P_H$  of the solution or to an oxidative change occurring in the pigment

That the chloroform solubility and dialysis properties vary with the solvent

That clinical and experimental evidence supports the view that bilirubin can be oxidized to biliverdin in the circulation, which pigment only gives the indirect reaction

It is felt that this view explains in a like manner the varying results obtained with the van den Bergh reaction. It does not in any way detract from the value of the reaction, but it seems to shed light on those not infrequent cases that do not fall in with the clinical expectations

It is offered as an explanation that in obstructive jaundice there is a constant absorption of bilirubin into the circulation from the distended bile channels—channels which are more or less long sealed tubes—in which oxidation cannot freely occur. It is interesting to record that as early as 1889 Hayercraft and Scofield observed the various changes that occurred in the constitution of the bile pigments found in the gall bladder. They came to the conclusion that reduction of biliverdin into bilirubin was a common phenomenon, as bile in the middle of the gall bladder was green and the bile near the bladder wall was orange brown. In consequence, bilirubin is present in just sufficient amount almost constantly in the circulation to give a direct diazo reaction. In the early stages of obstruction and when the obstruction is being relieved it is well known clinically how the van den Bergh reaction changes. In the early and late stages (when the icterus has been relieved) it may vary from a delayed to a negative direct. The icterus is not very deep, and oxidation may have been sufficient to transform the bilirubin into biliverdin. This change is well seen in the superficial tissues—a change which can take place quite as readily in the circulation.

Van den Bergh, in his work, says that he frequently came across a direct diazo reaction in some cases of icterus due to terminal heart failure and after a careful search no anatomical changes could account for any obstruction. Eppinger's biliary thrombi were not observed. The direct reaction here can be explained possibly by the lack of oxidation, bilirubin not being changed as readily into biliverdin as in those cases with an intact circulation.

The daily variations obtained in cases of catarrhal jaundice can be accounted for if one regards as the criterion of a direct reaction the presence of bilirubin in the circulation.

**BLASTOMYCOSIS** The Effect of Gentian Violet on the Organism of Blastomycotic Infection, Sanderson, E. S., and Smith, D. C. *Arch Dermat and Syph*, 1927, xvi, No. 2, p. 154

In vitro experiments with a recently isolated strain of blastomyces have shown that extremely dilute solutions of gentian violet (1:500,000) possess a marked inhibitory effect on its growth. It is possible that this dye may be utilized in the treatment of human beings who have this disease.

**SCARLET FEVER** Agglutinins in Human Sera for Scarlet Fever Streptococci Baumgartner, L. *Am Jour Pub Health* 1927, xiv, No 18 p 814

Agglutinins for streptococci isolated from acute cases of scarlet fever are found in the blood of apparently healthy and normal individuals

Of the 71.4 per cent whose sera did not agglutinate the scarlet fever streptococci 77.6 per cent had not had the disease

Of the sera from 188 individuals 28.6 per cent agglutinated scarlet fever streptococci. Twenty nine and six tenths per cent of the positive agglutinating sera came from individuals who had had scarlet fever previously

Of the individuals who had had scarlet 33.3 per cent had agglutinating bodies for scarlet fever streptococci in their blood

Counting only 3 and 4 plus agglutination reactions, 8.2 per cent and counting only 4 plus reactions, 2.0 per cent of the individuals whose sera agglutinated the scarlet fever streptococci had not had scarlet fever. Fifty per cent of this group had a history of tonsillitis and tonsillectomies

Maximum agglutination occurred in the serum dilution 1:80

Agglutination seemed to be specific if judged by the results of agglutination tests with three strains of streptococci of non scarlatinal origin

**GASTRIC TEST** Direct Examination of the Gastric Juice Gslambos A. *Arch Int Med* 1926, xxviii 6:4

The Rehfuus tube may be introduced into the fasting stomach and left there to induce gastric secretion which can then be fractionally withdrawn

The examination and study of gastric juice unmixed with food can thus be made possible, and the results used to complete the test meal examinations or as a substitute for them as the method gives a more exact account of the concentration and quantity of gastric juice secreted

Two types of gastric secretion are discriminated that of the normal stomach and that of the stomach affected with irritative secretory disturbance

In the normal stomach after fasting, even when the tube is left in place from ten to sixty or more minutes after introduction no gastric secretion can be obtained, either by drainage or suction

In the stomach affected by irritative secretory disturbance there is hypersecretion on fasting usually with hyperacidity, though normal acidity and occasionally anacidity may be noted. After a three to fifteen minute interval similar gastric juice may be obtained by suction. Occasionally, hypersecretion with anacidity is found in cases showing free hydrochloric acid and possibly later hyperacidity after the customary test meals

The amount of gastric juice secreted immediately after the insertion of the tube may be several times greater than the relative quantity accumulated during the night's rest. The acid concentration is, as a rule, much less increased

From one half an hour to one hour after the tube is inserted a phase of exhaustion of the mechanism of gastric secretion will intervene the quantity and concentration of the gastric juice being temporarily markedly reduced

This phase of exhaustion is characterized by a protective mechanism which produces a secretion with heavy admixture of mucus and regurgitated gall and also containing duodenal secretion

The first phase of exhaustion endures from ten to fifteen minutes. After an active interval of thirty to sixty minutes there will be a second period of exhaustion often lasting many hours

Information concerning the motility of the stomach can be obtained through the use of this test, at the same time the gastric juice is being withdrawn for study. For this purpose it should be performed when the stomach has just been emptied after the administration of a test meal e.g. two hours after the test breakfast



**TISSUE TECHNIC** A Rapid Method for the Silver Impregnation of Reticulum Foot  
N C and Menard, M C Arch Path and Lab Med, 1927, iv, 211

In detail, the method is as follows. Sections of Zenker fixed material are cut to from 5 to 7 microns in paraffin, care being taken to affix them smoothly and flatly to the glass and to dry them out thoroughly in the paraffin over for at least twelve hours before proceeding with the impregnation. The sections are cleared of paraffin in the usual manner, being run through xylol and alcohol of descending percentages into mahogany brown alcoholic iodine, where they stay for five minutes. They are washed and bleached in 5 per cent sodium thiosulphate, washed again and the chromium salts of the Zenker's fluid removed by five minutes' treatment in 0.25 per cent aqueous potassium permanganate and ten minutes' in 5 per cent aqueous oxalic acid, with a rinse between. They are washed first at the tap and then in distilled water.

The sections are next treated with silver ammonium carbonate, which must be prepared fresh for each batch of slides, as it does not keep well. To 10 cc of a 10 per cent aqueous solution of silver nitrate, is added 10 cc of a saturated solution of lithium carbonate, distilled water being used for both. The resulting heavy white precipitate of silver carbonate is allowed to settle, and the supernatant fluid poured off. The precipitate is then washed several times in from 25 to 50 cc of distilled water, being allowed to settle each time and the supernatant fluid being decanted. After 25 cc of fresh distilled water is added this washed precipitate is almost dissolved in strong ammonia water, which is added drop by drop while the container is shaken continuously. The precipitate turns grayish brown as the ammonia is added, about 8 to 15 drops of the ammonia will be needed, depending on its concentration. Extreme care must be taken not to overstep the end point of solution, for it is better to allow a few granules of precipitate to go undissolved. The entire solution is then made up to 100 cc with distilled water and heated to about 50° C, or until it becomes uncomfortably warm to the touch. The slides are immersed in this solution and placed in an incubator at 37° C for from ten to fifteen minutes, until they turn a yellowish gray. The temperature of the bath will remain between 40 and 50° C under these circumstances. Care must be exercised lest the sections become detached from the slides in this warm, alkaline bath, if they have been properly affixed and dried they should not come away.

After being rinsed quickly in distilled water, they are placed in 20 per cent neutral formalin (8 per cent formaldehyde) for two minutes, in which they become dark brown. They are washed well at the tap and transferred to a bath of 1:500 gold chloride in water for two minutes, when they fade to a delicate violet gray. After washing, the superfluous silver salts are removed and the sections fixed in 5 per cent aqueous thiosulphate of soda and then washed. The reticulum is now impregnated and the sections may, if it is desired, be run through ascending percentages of alcohol into xylol and mounted in Canada balsam. To furnish a background, a more lively collagen stain and a more detailed demonstration of the nuclei, a combination of Harris' hematoxylin and van Gieson's stain should be superimposed on the silver impregnation.

- a From three to five minutes in Harris' hematoxylin, wash at tap till blue
- b Forty five seconds in van Gieson's picric acid and fuchsin stain
- c Run immediately into 95 per cent alcohol, avoiding water, and follow with absolute alcohol and xylol, mounting in Canada balsam

The finished, counterstained sections will, as in Foot's modification of the Bielschowsky-Maresch method, show the reticulum in sharply defined black lines, the collagen in vermilion to crimson, the nuclei in reddish brown and the cytoplasm and muscular tissue in yellow. Erythrocytes will vary from yellow to greenish gray. We have tried many variations on the foregoing method, such as changing the length of impregnation time and the concentration of the silver ammonium carbonate. It was found that as little as 2 cc of silver nitrate to a like amount of lithium carbonate, diluted to 100 cc with distilled water, would give excellent results, the most constant and satisfactory impregnations, however, were obtained by the method detailed, which may be briefly summarized as follows:

1 Remove paraffin from sections of Zenker fixed tissue in the usual way and treat with alcoholic iodine and 5 per cent sodium hyposulphite to remove the mercury salts Wash at the tap

2 Treat for five minutes with 0.5 per cent aqueous potassium permanganate and for ten minutes with 5 per cent aqueous oxalic acid, with a wash between Wash well at tap and follow with a wash in distilled water

3 Treat for ten or fifteen minutes with silver ammonium carbonate at about 45 C

4 Wash in distilled water and reduce in 20 per cent neutral formalin for two minutes Rinse well at tap

5 Tono in 1:500 gold chloride solution for two minutes

6 Wash and fix for two minutes in 5 per cent sodium hyposulphite

7 Wash well and stain for from three to five minutes in Harris' hematoxylin and then soak in running water till blue

8 Stain for forty five seconds in van Gieson's picric acid acid fuchsin

9 Wash in 95 per cent alcohol and run through alcohol of ascending percentage into xylol, mounting in Canada balsam

#### STAPHYLOCOCCAL HEMOLYSIN A New Method of Preparing Staphylococcal Hemolysin Bigger J W Boland, C R and O Meara R A Q Jour Path and Bact, 1927, xxx, 271

A new method of preparing staphylococcal hemolysin is described It consists in suspending staphylococci from a solid medium in saline and freeing the fluid from cocci by centrifuging

The lysin so prepared is purer than any previously described since it is free from the various adventitious substances present in broth cultures

It has a high titre and a M.H.D. of 0.000007 cm for sheep cells has been recorded where the total volume in each tube was 2.0 cc and the amount of cell suspension present similar to that used in the Wassermann test

The lysin acts on sheep cells and human cells but better on the former than on the latter It acts at cold room, air and body temperatures and the highest titres are obtained by incubation at 37 C followed by exposure to air or cold room temperature

Twenty four strains of *Staphylococcus aureus* were tested and sixteen of these were found to produce this lysin In some instances an organism freshly isolated did not and after a few subcultures did, produce the lysin

This lysin is probably identical with the lysin produced in broth cultures

By using this lysin, hemolysis in blood agar plates has been produced, identical with that produced by a colony of a hemolytic *Staphylococcus aureus*

The lysin is very thermostable, the titre being only reduced to about one half by an exposure to 100 C for half an hour

The lysin can be obtained in dry form by evaporation and remains unaltered in activity in this condition for months

It is insoluble in alcohol ether chloroform or acetone

The lysin acts best in a medium with a slightly alkaline reaction

It has not been found possible to produce a definite antibody to this lysin

#### MOSQUITO IDENTIFICATION Rapid Determination of Anopheles Larvae in a New Medium Zetek J Jour Trop Med, 1927, vii No 4 247

All the apparatus required is (a) A hand lens (the ordinary 10 X is sufficient though a portable pocket microscope giving a magnification of 32 X is preferable) (b) a micro culture slide, such as is used for drop cultures 1 inch by 3 inches of thick polished plate glass, with flat bottom, the depression 3 mm deep by 16 mm diameter inside of bottom smooth but not polished (c) an ordinary medicine dropper with the tip broken off so that it can take up a larva with ease

The larvae are examined alive. A solution is made of Klum or any other milk powder, about the consistency of milk, and this is placed in the cavity of the culture slide, almost to the level and a larva is placed into this milk solution by means of the dropper. The larvae when placed in this solution come to the surface and remain very quiet, but all that is seen are the palmar hairs, the antennae and moving mouth brushes.

# **EPITHELIOMA Lattice Fibers and their Diagnostic Value in Epithelioma, Way S C** Arch Dermat and Syph, 1927, 111, No 1, 25

The author suggests that the determination of transition from a simple type of growth to one in which malignancy is becoming evident and also the comparative degree of malignancy may be determined by a study of the "lattice fibers" (immature connective tissue fibrils).

An epithelioma may be said to be benign when it is entirely surrounded by a dense, thick network of lattice fibers, provided that the cancer cells do not penetrate into the surrounding fibers and there is a definite line of demarcation between the two.

An epithelioma of the ordinary type may be said to be malignant when the lattice fibers surrounding the cancerous growth are few or practically absent, and when, instead of showing a sharp line of demarcation between the two, the cancer cells penetrate into the bordering lattice fibers.

They may be demonstrated by the following technique.

- 1 Fix the material preferably in solution of formaldehyde in a 10 per cent dilution and embed in paraffin. The sections should be cut thin (from 4 to 6 microns) and mounted on albuminized glass slides.

- 2 Run the section down through xylol, absolute, 90 per cent, and 70 per cent alcohol to water, transferring it at once to a 2 per cent silver nitrate solution, where it should remain for from twenty-four to forty-eight hours.

- 3 Wash briefly in water and then allow the slide to remain one-half hour in a solution which is prepared as follows. To 25 cc of a 10 per cent silver nitrate solution add 25 drops of a 40 per cent sodium hydroxide solution. Stirring constantly, slowly add aqua ammonia by means of a pipette until the precipitate almost disappears, filter carefully and add distilled water up to 100 cc.

- 4 Wash as rapidly as possible in distilled water and permit the section to remain one-half hour in solution of formaldehyde in a 5 per cent dilution. As soon as the section is placed in solution of formaldehyde, it should instantly turn black.

- 5 After washing a few seconds, place section in a 1 per cent gold chloride solution for one hour.

- 6 Wash in distilled water and then two minutes in a 5 per cent solution of sodium hyposulphite.

- 7 Wash section thoroughly in water from four to six hours, then counterstain with picrofuchsin, as in Van Gieson's method, if necessary.

- 8 Run the section up through 70 per cent, 90 per cent and absolute alcohol to xylol and mount in balsam. The lattice fibers are stained a deep black.

# **ASTHMA The Non Specific Diagnosis of Allergic Asthma, Feinberg, S M, Taub, S J, and Unger L** Annals Clin Med, 1927, 1, 1124

The authors relate investigations which tend to corroborate the observation of Van Leeuwen that all cases of allergy, regardless of the sensitizing agent, react to an extract of human dander and that a non specific diagnosis of allergy may thus be made by a single skin test.

The preparation of the extract was as follows.

To 1 gram of fresh dander obtained from healthy scalps were added 100 cc of physiologic salt solution and allowed to stand twenty-four hours at room temperature. The physiologic salt solution was then sterilized by Berkefeld filtration and enough phenol for a final

concentration of 0.5 per cent was added. After testing for sterility the extract was ready for use. However it is necessary to test each specimen of extract as occasionally one will be unfit for use because it will give positive reactions in normal individuals.

The patient to be tested is injected intradermally on the flexor surface of the forearm with 0.02 c.c. of this extract and using for a control the same chemical solution to which no dander has been added. A positive reaction consists of an wheal measuring 0.5 cm. or larger with surrounding erythema and usually occurring in five to ten minutes.

**BLOOD CHEMISTRY** A Colorimetric Method for the Determination of Acetone Bodies in Blood and Urine. Behre J. A. and Benedict S. R. Jour. Biol. Chem. 19:6 1906 2:487

#### REAGENTS

For the Removal of Sugar and Other Interfering Substances by the Van Slyke Method (with Modified Concentrations). Copper sulphate 40 per cent solution or powdered in a mortar. Calcium hydroxide 20 per cent suspension or dry.

For the Oxidation of B-Hydroxybutyric Acid by the Shaffer-Hubbard Method. 10 per cent concentrated sulphuric acid. 0.1 per cent potassium bichromate solution.

For the Removal of Blood Proteins by the Folin-Wu Method. 10 per cent sodium tungstate solution. Two thirds normal sulphuric acid.

For the Colorimetric Determination of Acetone. Sodium hydroxide 32 per cent solution. Salicylic aldehyde.

Kahlbaum's technical salicylaldehyde proved very satisfactory as did also Eimer and Amend's high grade product labelled "Acid salicylic synthetic (salicylic aldehyde)".

#### STANDARD ACETONE SOLUTIONS

**Stock Solution.** This contains 0.1 mg. of acetone per c.c. It is most easily prepared from a solution containing 1 c.c. of acetone in 1 liter of water whose actual acetone content by weight has been determined by an iodine titration. The stock solution should be prepared from this to contain 0.1 mg. of acetone per c.c. This solution can be kept for about a month without deterioration.

**Standard Solution.** By 1:10 dilution of the stock solution a standard solution containing 0.01 mg. per c.c. is prepared for use in the actual determination. It is best to make up this dilute solution every 2nd day and to keep it well corked when not in use.

#### DETERMINATION OF ACETONE BODIES IN URINE

##### Preformed Acetone and Acetone from Diacetic Acid

(A) If B-hydroxybutyric acid is not to be determined. Such a volume of urine as will contain about 0.1 mg. of acetone (usually from 2 to 50 c.c. as required) is transferred to a 100 or 150 c.c. distilling flask the volume made up to about 75 c.c. with distilled water, and 3 or 4 drops of sulphuric acid diluted 1:1 are added. The flask is tightly fitted with a cork stopper and connected with a water-cooled condenser. The condenser is provided with a bent glass tube which has been drawn out long enough and to a sufficiently small diameter to reach to the bottom of a 25 or 50 c.c. volumetric flask and which dips below the surface of a minimum amount of water in the flask. None of the connections should be of rubber. Rubber stoppers covered with tin foil or cork stoppers often renewed, can be used. The preformed acetone and acetone from diacetic acid are then distilled into the 25 or 50 c.c. flask. Except when very large amounts of acetone are present a distillation to 25 c.c. gives good results. When the distillate has almost reached the volume desired the bent tube is disconnected and washed out with a few drops of water and the distillate made up to volume and mixed. 5 c.c. of the distillate are transferred to a test tube and exactly 5 c.c. of a 32 per cent solution of sodium hydroxide and 10 drops of salicylic aldehyde are added. Standards are prepared in test tubes at the same time from the dilute (0.01 mg. per c.c.) acetone solution. By using from 0.5 to 5 c.c. of this solution a range of standards containing from

0.005 to 0.05 mg can be made. Unless the approximate acetone content of the unknown solution is known, standards containing 0.005, 0.01, 0.02, 0.03, and 0.05 mg had best be made. In each case the volume of the standard solution must be made to 5 cc. Exactly 5 cc of 32 per cent sodium hydroxide and 10 drops of salicylic aldehyde are also added to each of the standard tubes. The contents of the tubes are mixed by side to side shaking and the tubes then immersed in a boiling water bath for from three to five minutes. If the salicylic aldehyde does not dissolve easily the tubes must be shaken until solution is effected. After the heating the tubes are removed and allowed to cool, the solutions filtered, and colorimetric comparison is made. The standard used should be of such a concentration that the unknown solution gives a reading between 11 and 19 mm with the standard set at 15 mm.

In making the calculation the following formula may be used

$$\frac{r}{y} \times \frac{p}{s} \times \frac{t}{5} \times 100 = \text{No of gm of acetone in 100 cc of blood or urine}$$

$r$  = reading of standard

$y$  = reading of unknown

$p$  = gm acetone in standard

$s$  = cc of blood or urine used

$t$  = cc, volume of total distillate

(B) If B-hydroxybutyric acid is also to be determined. If B-hydroxybutyric acid is to be determined in addition to acetone, sugar and other interfering substances must be removed, even from normal urine, before any distillation is made. For this the urine is treated with copper sulphate and calcium hydroxide according to the Van Slyke procedure. In order to keep down the volume of solution to be distilled, however, the urine is diluted 1:5 instead of 1:10, using 1 volume of urine, 1 volume of 40 per cent copper sulphate solution, and enough of a 20 per cent suspension of calcium hydroxide to make the reaction alkaline to litmus (probably 1 volume). The whole mixture is then made up to 5 volumes. If the urine is very low in acetone bodies the copper sulphate can be powdered in a mortar and both this and the calcium hydroxide added in powdered form. The copper sulphate should be dissolved before the calcium hydroxide is added. The mixture must be shaken very thoroughly and allowed to stand for one half to three fourths of an hour, with occasional shaking. It is then filtered and a volume of the filtrate equivalent to from 2 to 50 cc of urine (depending on the acetone content) placed in a 300 cc distilling flask and made acid with 3 or 4 drops of sulphuric acid (diluted 1:1). The volume is made up to about 75 cc, the flask fitted with a dropping funnel, and connected to a water cooled condenser, and the distillation and determination of acetone and diacetic acid carried out as described under (A).

#### OXIDATION OF B-HYDROXYBUTYRIC ACID BY THE HUBBARD SHAFER METHOD AND ITS DETERMINATION AS ACETONE

After distillation of the preformed acetone, a 100 cc receiving flask is substituted for the 25 cc flask, the residue in the distilling flask is brought to a boil, and 30 cc of half concentrated sulphuric acid and 20 cc of 0.2 per cent potassium bichromate are added gradually through the dropping funnel while a slow distillation goes on. Fifty cc more of the bichromate are added after ten minutes and 50 cc more after another interval of ten minutes. The process differs from the Hubbard method only in that the distillation is made very slowly and the volume of distillate kept down to 100 cc. The distillation should occupy at least thirty minutes. When the distillation to 100 cc is almost complete the receiving apparatus is again disconnected, the bent tube washed down with a little water, and the distillate made up to 100 cc and mixed. Acetone is determined colorimetrically in 5 cc of the distillate as described above under (A).

#### DETERMINATION OF ACETONE BODIES IN BLOOD

##### Preformed Acetone and Acetone from Diacetic Acid

The blood proteins are precipitated by the regular Folin Wu method (7), making a dilution of the blood of 1:10. From 10 to 100 cc of the filtrate, depending on the acetone

content are transferred to a 300 cc distilling flask 3 or 4 drops of concentrated sulphuric acid diluted 1:1 added the volume made up to 50 to 75 cc and distillation carried out as described for the determination in urine. The distillation is made into a 20 cc receiving flask or graduated test tube unless the acetone content of the amount of blood used is known to be high (above 0.5 mg) in which case the distillation is made into a 25 cc flask or graduated tube. If the amount of acetone in the filtrate used is known to be 0.1 mg or more, the distillation may be made to 50 cc. In any case the distillation is stopped just before the desired volume has been reached the distillate is made up to volume and 5 cc of the distillate is heated with alkali and salicylic aldehyde cooled and read in a colorimeter as described for the urinary determination.

#### DETERMINATION OF ACETONE FROM B-HYDROXYBUTYRIC ACID IN THE BLOOD

1 If the actual volume of filtrate used is known to contain 0.1 mg of B-hydroxybutyric acid as acetone or more the distillation is carried out as described for the determination in urine the volume of the distillate being kept within 100 cc and finally made up to 100 cc and the colorimetric determination being made upon 5 cc of the distillate as described above under (A).

2 If the volume of filtrate used is expected to contain less than 0.1 mg the distillation is carried out without regard to the volume of distillate collected and after the thirty minute period the distillate is redistilled into a 20, 25 or 30 cc volumetric flask (or graduated test tube) according to the amount of acetone expected. In any case the distillate is made up to volume and the acetone content of 5 cc of the distillate determined as described above.

The formula given in the section on urinary determination under (A) may be used for all the calculations.

## REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,  
Richmond, Va

### *The Psycho-Pathology of Tuberculosis\**

DR MONROE states that there is no disease in which the mental and moral characteristics of the patient are so profoundly modified and with which the psychoneuroses are so constantly associated, as chronic pulmonary tuberculosis. Although the profession may have paid too little heed to the psychologic problems so often presented by those who suffer from pulmonary tuberculosis, these problems have not escaped the notice of certain fiction writers who have portrayed the morbid psychic state characteristic of so many persons with tuberculosis. The author mentions some of the outstanding historical examples.

Chapters are devoted to the psychoneuroses of latent or incipient tuberculosis, of the middle stage of tuberculosis, of advanced tuberculosis, to phthisical insanity. There is a chapter on the sexual factor in tuberculosis and three most interesting ones on tuberculosis and genius. The last chapter deals with psychotherapy in the treatment of the disease. Among geniuses with tuberculosis we find names such as Robert Louis Stevenson, Keats, Aubrey Beardsley, John Addington Symonds, DeQuincy, John Ruskin, Ralph Waldo Emerson, Goethe, Schiller, Balzac, Jane Austen, John Locke, Laennec, Trudgen, and Thomas Hood. The biographic references and descriptions referring to these men of genius are of greatest interest whether one's primary interest be in the subject of tuberculosis or not.

### *Actions and Uses of the Salicylates and Cinchophen in Medicine†*

THIS contribution has a strictly utilitarian value to the clinician. Salicin was found in willow (*salix alba*) by Leroux in 1827. Salicylic acid was first prepared in 1838. The compounds of this drug are absorbed with remarkable facility, salicylic acid being absorbed through the intact skin and many of the salicylates through the nasal and oral mucosa, the rectum, the vagina, the stomach, and with particular ease through the intestinal tract. After absorption salicyl is found in nearly every secretion, fluid and organ of the body. It appears within four minutes in the saliva, ten minutes in the urine, and thirty minutes in the bile. It is even recoverable from the sweat. In the presence of inflammation salicyl is found in both exudates and transudates and in the inflamed tissues. However the alleged selectivity of salicylates for inflamed joints apparently does not exist, for it is present in no higher percentage in joint fluids than in the circulating blood. If anything, the inflamed membranes of the synovial act as barriers to their diffusion. The suggestion that salicylates act beneficially in rheumatic fever on account of the liberation of salicylic acid resultant on the high carbon dioxide content of the inflamed tissues has not been borne out experimentally. Its exact mode of action is not yet known.

While salicylate appears in most of the secretions it passes out of the body chiefly through the urine. It is absorbed so rapidly that little appears in the feces. Complete

\*The Psycho-Pathology of Tuberculosis By D G Macleod Munro M D C M M R C P  
Cloth 92 pages Oxford University Press American Branch New York 1926

†Actions and Uses of the Salicylates and Cinchophen in Medicine By B J Hanzlik  
M D Professor of Pharmacology Stanford University School of Medicine Cloth 200 pages  
Williams and Wilkins Baltimore 1927

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

elimination may take place in twelve hours but ordinarily this requires from twenty four to forty eight hours. At the same time there is some actual destruction of salicylate within the body. Fever seems to increase its destruction.

Salicylates increase nitrogenous metabolism. Total nitrogen and uric acid in the urine are increased after one dose. The increase in protein decomposition averages from 10.6 to 13.4 per cent above normal. This is compensated for by a loss during the after periods of treatment. Salicylates increase the excretion of uric acid. This of course has its application in the treatment of gout. Sodium salicylate increases uric acid excretion from 40 to 50 per cent. The mechanism of increased uric acid excretion seems to be the same for both salicyl and cinchophen and is concerned with the kidneys rather than with some obscure change in the metabolism or mobilization of the urates in the tissues. It is believed that salicylate lowers the threshold of the kidney for uric acid. Salicylate appears not to influence uric acid excretion as favorably when there is extensive renal damage.

Dr. Hanzlik discusses the pharmacologic action of these drugs in the blood, gastrointestinal tract, kidneys, circulation and respiration. In the clinical section of the volume there are chapters devoted to antipyretic action in different clinical fevers, its action in rheumatic fever and in other clinical conditions. Other chapters deal with toxicology and methods of administration.

### *A Textbook of Psychiatry for Students and Practitioners\**

TOO many textbooks of psychiatry present exhaustive descriptions of the psychopathies as clinical entities with little or no attention to the social aspects of the victim. In other words the discussions are of these diseases as they are observed in the environment of the psychopathic ward or insane asylum and not in the environment of the community. Obviously the first environment is that of the community and it is here that the psychopath finds his greatest problems. The aim of these authors has been to present psychiatry as a living subject with important relations not only to general medicine but to the social problems of everyday life. The authors draw particularly upon the biologic viewpoint of Adolph Meyer which regards mental illness as the cumulative result of unhealthy reactions of the individual mind to its environment and seeks to trace in a given case all the factors that go to the production of these reactions. Mental illness is an individual affair. Its symptoms have little meaning apart from the setting in which they occur. Clinical syndromes, while interesting are not of the first importance. What is wanted is an understanding of the patient as a human being and of the problems which he is meeting, in a morbid way with 'his symptoms'.

Aside from considerations of classification, etiology, methods of examination, symptomatology, general psychopathology, and chapters on the individual groups of psychoses there are sections devoted to historical review of the care and treatment of mental illness, occupational therapy and the relations of psychiatry and law.

### *Clinical Neurology for Practitioners of Medicine and Medical Students†*

A TEXTBOOK of neurology written more for the internist and general practitioner than for the neurologist, on the basic idea that it is the practitioner who sees the patient early when he is still in the hopeful phases of his illness, while the neurologist usually witnesses the terminal stages which are interesting but usually quite hopeless.

*A Textbook of Psychiatry for Students and Practitioners*. By D. J. Henler, an M.D. FRF.P.S. Physician Superintendent, The Glasgow Royal Mental Hospital and R. D. Gillespie, M.D. D.P.M. Physician for Psychologic Medicine Guy's Hospital, London. Cloth. 306 pages. Oxford University Press, New York.

†*Clinical Neurology for Practitioners of Medicine and Medical Students* largely based on the book by Professor Hans Curschmann, Rostock, Germany, with a free translation with changes and additions by Edward A. Strecker, A.M. M.D. Professor of Nervous and Mental Diseases, Jefferson Medical College and Milton K. Meyers, B.S. L.L.B. M.D. Neurologist at the Northern Liberty Hospital, Philadelphia. Cloth. 410 pages. Blakiston, Philadelphia, 1927.



### *Hypotension\**

THOSE factors responsible for hypotension are in the main the same factors which are responsible for the maintenance of normal blood pressure. These are, (1) the force of the cardiac contraction, (2) the condition of the vessel walls, (3) the peripheral resistance, and (4) the blood volume and physical state of the blood itself. The first is of course a factor in myocardial decompensation while the second, that is the condition of the larger vessel walls, usually plays little part in the causation of hypotension. The third and fourth factors are often of great importance.

We may briefly summarize the average normal blood pressure changes in man as follows: the systolic blood pressure of newborn infants runs between 45 and 55 mm Hg, the diastolic about 15 points lower. It increases daily after birth until on the tenth day it reaches an average of about 78 mm, systolic. The greatest rise takes place during the first three days. From ages three to ten the average systolic pressure runs from 90 to 100 mm. Faber and James found hypotension to be rather common in children, particularly in asthmatics. From eleven to fourteen years the average systolic runs in boys around 107, diastolic 71, in girls 106/63. Up to 11 years the mean systolic pressure rises uniformly with age. After puberty a more rapid increase develops. The average adult level is reached in both sexes between the ages of seventeen and twenty. In the early adult years there is evidence of a slight lowering of pressure perhaps slightly more marked in men than in women. Little support is found for the statement that the pressure in healthy persons increases as the years go by. It remains practically constant until age forty after which the figures for healthy women are a little higher than for men. The total rise due to age and increasing weight is usually not more than 15 mm. The relatively wide variations from these normal averages in apparently normal individuals is still to be explained. It seems probable that heredity plays some part. Race is also a factor, the systolic pressure for Chinese, for example, being 20 to 30 mm less than of Caucasians. The pulse pressure, however, is the same.

Hypotension does occur in otherwise apparently healthy individuals. Indeed hypotension without other evidence of disease appears to be a desirable attribute. Fisher of The North western Life Insurance Company found that in their hypotension series the death rate was only 35 per cent of the expected mortality as contrasted with their general mortality of 80 per cent of the expected. Muhlberg of the Union Central Life Insurance Company remarks that "There appears to be no doubt of the fact that a low blood pressure past the age of fifty unassociated with any organic lesions to account for this low blood pressure is the best criterion that we possess that the individual will live beyond his normal expectancy." It has been remarked that often the hypotensives who in their earlier adult years have some symptoms of asthenia, later, when their blood pressures rise to around the usual normal are in much better physical and mental condition than they were formerly or than are other adults of the same age.

Barach, in a study of 655 healthy, young adults found 23 with systolic pressures between 100 and 110 and 7 between 90 and 100. Thus, 45 per cent showed hypotension. In a series of 31,596 recruits examined at Camp Sherman 55 per cent showed hypotension without cardiovascular lesion or other organic disease. Of 1,100 freshmen examined at the Carnegie Institute in 1924, 25 per cent presented hypotension. Alvarez, examining 6,000 men in the University of California, found 22 per cent of hypotensives. We may say that hypotension, systolic pressure below 110, appears to occur in about 3 per cent of apparently healthy, normal white adults.

The author devotes considerable attention to blood pressure findings in the various diseases and to the effect of drugs on the blood pressure. His discussion of so called essential hypotension is thorough and at the same time concise. There are nearly as many theories regarding the cause of essential hypotension as there are writers on the subject. Friedlander adds a new theory. To the reviewer, no one of the theories appears to be convincingly complete.

The medical profession has needed a comprehensive review of the literature on hypotension and Dr. Friedlander furnishes this very satisfactorily.

\*Hypotension. By Alfred Friedlander. Professor of Medicine. College of Medicine. University of Cincinnati. Cloth. Pages 216. Williams and Wilkins. Baltimore. 1927.

### *A Bipolar Theory of Living Processes\**

DR CRILE'S theories are always interesting. They usually stir up considerable discussion. He usually tackles a pretty heavy subject such as the nature of life. The present volume is presented as a culmination of his studies and understanding of the process of living and is based upon many years of experimental study along chemical, cytological, and biophysical lines.

His theory and postulates are briefly as follows. Living organisms are bipolar electric mechanisms. Electricity is a constant phenomenon of living processes. The application of electricity to the muscles or the glands or their nerve supply will cause them to perform their natural functions. The materials of which animals are constructed are specifically adapted to electrical processes. In structure and function the unit cells which drive the organism are adapted to fabricate store and discharge electricity. This is also true of protoplasm itself. The organism as a whole is a bipolar electric mechanism bearing the pattern of the unit cells and the unit cells are constructed on the pattern of the atom. The normal and pathologic phenomena of man and animal may be interpreted in electrical terms. The unit cell of the body is a bipolar mechanism, the nucleus and the surrounding protoplasm carrying opposite electrical charges so that the cell acts very much as a battery.

On the basis of Dr Crile's theory which he develops in great detail he suggests a bipolar interpretation of such clinical conditions as anesthesia, hyperthyroidism, infection, cancer, memory, reproduction and heredity.

Theories are but steppingstones necessary for progress but of little value as long as we stand still on the one theory. New theories must rise up to take their place as we advance. As a steppingstone Dr Crile's monograph should be of the greatest interest to all students of life.

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### *International Clinics†*

INTERNATIONAL Clinics through the many years of its existence has become an institution. Such a wide variety of subjects are considered that no one man will find an equal interest in every contribution. However the papers are primarily of clinical interest and the wide variety of subjects adds to rather than detracts from the readability.

In the five volumes under review one may find an authoritative expression on almost any subject in clinical medicine. There are several most enjoyable contributions touching upon the history of medicine. A brief biographic review of the life of Sir Clifford Allbutt by Sir Humphrey Rolleston is rather disappointing. It reads rather too much like a calendar enumerating dates and events and leaving us with the feeling that we have gained no insight into the great man himself.

Dr Kisskalt of Zurich has an address on the origin and passing of epidemic diseases. This is not alone of interest to the epidemiologist for it is a most absorbing historical review of the course of the great epidemics in history.

"Medicine from the standpoint of history is interesting. The part that medicine plays in archaeological studies and the value of archaeological studies to our knowledge of the history of medicine is well brought out. Every physician at one time or another starts the reading of medical history. Much depends upon the character of the first book which falls into the doctor's hands. If it be dull a mere repetition of names and dates, a mere series of discoveries in anatomy and physiology then for that individual medical history will be dull always.

John R. Oliver contributes a paper on Greek medicine. This author has gone into the greatest detail in searching out those interesting facts of early Greek medicine which usually do not appear in historical reviews. How little indeed do we actually know of Hippocrates. Most of the Hippocratic writings have probably been from the pens of his

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\* *A Bipolar Theory of Living Processes*. By George W. Crile. Cloth. 400 pages. Macmillan, New York. 1916.

† *International Clinics*. Thirty-sixth series, volumes three and four, September and December, 1916; thirty-seventh series, volumes one, two and three, March, June and September, 1917. Lippincott, Philadelphia.

pupils Of this one man whose influence has been so tremendous, whose ideals are still powerful today, we know almost nothing There are only three contemporary references to Hippocrates, one by Aristotle and two by Plato Aristotle tells us that in his day Hippocrates was already known as "The Great Hippocrates" Plato says that Hippocrates came from the island of Cos, that he was an asclepiad and that he taught medicine for money Beyond these short, contemporary references all of our acquaintance with this great man has come from others who have lived after his day

In the series under review cardiovascular diseases receive their full share of consideration L F Bishop of New York reports a series of seven patients with blood pressure of 200 or more whom he has followed for at least ten years His general principle of treatment has been to teach them that blood pressure is in a sense compensatory and it need not necessarily be brought down However, he carefully supervises daily routine He epitomizes his methods of supervision as "exercise, diet, and castor oil" This contribution is well worth the reading

An article on "hypertension and hypertonia—hypotension and hypotonia" by Dr J Pal, Professor of Medicine in the University of Vienna is rather disappointing It tells us nothing new It was prepared as a lecture to the Interstate Postgraduate Assembly of North American Physicians in Europe Many others of these lectures to the Interstate Postgraduate Assembly have been published in these volumes and some are very instructive

J J Walsh of New York describes a case with extrasystoles of forty years duration He has followed the man to age sixty and the victim is still quite well He describes several other cases of functional extrasystole thereby emphasizing the fact, which should be better known to all, that premature beats are often of no serious import

Percy B Davidson of Boston writes of Common Digestive Syndromes Encountered in Cardiovascular Disease Too often we forget that the cause of acute or chronic abdominal symptoms may be entirely above the diaphragm Cardiovascular disease, especially the arteriosclerotic type, may present the picture of an acute abdomen, as in coronary thrombosis, or of common relatively chronic digestive syndromes such as carcinoma of the stomach, peptic ulcer, appendicitis, and cholecystitis A patient who has been treated for months as a gastrointestinal case who after visiting another physician is diagnosed as a myocardial condition and whose symptoms clear up as by magic following rest in bed and digitalis is a source of deep embarrassment to the first physician

Bishop again contributes a most instructive paper on a heart block clinic This is abundantly illustrated with case reports, orthodiagrams and electrocardiograms

Gastric and duodenal ulcer come in for their share of discussion René A Gutmann of Paris has a practical paper on the medical treatment of uncomplicated gastric ulcer His review of both medicinal and dietary treatment should be at the elbow of the man who treats his ulcers incidentally, for it has many helpful suggestions for the individual case

John Phillips of Cleveland has a paper on a similar subject He brings out that ten years ago surgeons were doing their best to prove the superiority of surgery in ulcer, while the internists were doing likewise for medicine Now the two groups have gotten together and their first interest is the most appropriate form of treatment for the individual case Phillips reviews those types which are amenable to medical or surgical treatment, then devotes considerable attention to diet and to medicinal therapy His outlines for instructions to the patient are very helpful

Stanley P Reimann and L Snellbaker of Philadelphia have made a clinical study of gastric function following operations on the stomach We know much of the normal stomach and considerable of the stomach afflicted with ulcer We need more information on the physiology of the stomach which has been altered by extensive surgery This contribution helps our knowledge in this field but much additional work remains to be done

There are only two contributions on allergy in the volumes under review but they are both by authorities and well worth the reading One is by Nathan Schiff of New York who probably has done more on the nonspecific protein treatment of asthma than any other man in the United States The other by Storm Van Leeuwen of Leiden, Holland, is in a sense an appendix to his earlier monograph Van Leeuwen believes that many sensitizations are

to moulds and his method of eliminating these from the air from pillow mattresses and the like is carefully detailed. He also carefully analyzes his results. No one in this country so far as the reviewer knows has done as much work along this line as Van Leeuwen.

Other interesting contributions are on colitis, endemic goiter in Switzerland, liver function, novasurol in cirrhosis of the liver, amebiasis, acedosis in children and in diabetes, and vitamins. There are several good neurologic subjects.

### *Applied Physiology\**

IT IS now generally recognized that the science and art of medicine can only find a sure foundation on a sound knowledge of physiology. Applied physiology may be regarded as a branch of pathology inasmuch as it deals with the physiology of disease. Morbid processes have never received at the hands of pathologists the attention they deserve. Morbid anatomy and bacteriology have claimed most of the attention of workers.

"The book should be specially valuable to students who have passed through their courses in Physiology and are entering upon their work in the wards and various departments of the hospital. A presentation of the facts of physiology in close connection with their medical bearings would in itself be of value but Dr Wright's book does more than this. It presents many facts and theories on the borderland of physiology and medicine which the student would have great difficulty in digging out for himself. —Swale Vincent

### *Lectures on Internal Medicine†*

THIS volume is a series of four lectures delivered by Dr. Faber while in the United States in 1926. The first is on achylia gastrica. He discards the functional theory and the theory of congenital abnormality or aplasia and concludes from his researches that most of the so-called achylia is due to destruction of the acid-secreting glands through a pyrenchymatous disease of the stomach which involves not only the glandular substance but usually more of the stomach wall—which may be due to injury from substances taken into the stomach but is perhaps more often associated with bacterial or toxic action and is quite analogous to parenchymatous disease of the kidneys and other organs. Having developed a technique by which he can make postmortem examinations before the rapid postmortem changes in the stomach have occurred, he has been able to demonstrate inflammatory changes.

Dr. Faber believes that achylia is the most frequent but not the only cause of pernicious anemia. He considers that the latter disease is not an etiologic or pathogenetic entity. A continually occurring intoxication is the most natural explanation and in the majority of cases it is an intestinal intoxication by some form of protean toxin.

Faber has never seen a case of benign glycosuria with normal blood sugar curves (due usually to a lowered threshold of sugar excretion) develop into true diabetes.

In a sketchy historical outline of medical therapy which is full of interesting information Faber describes how extensively blood letting was practiced in the middle ages and even much later. In 1857 thirty-three million leeches were imported into France. It has been calculated that in the year 1800 by means of leeches 85,000 liters of blood were tapped in Parisian hospitals. A woman who died at thirty-one was during the last fifteen years of her life bled 1309 times.

The reason for bleeding of course was the removal of noxious humors. In acute diseases such as pneumonia the buffy coat of fibrin which formed at the surface was considered proof of the presence of too much phlegma or mucus in the blood. The first man to take a positive stand against promiscuous blood letting was Pierre F. Alexandre Louis. His communication raised a storm of protest. The protest was chiefly that a numerical

Applied Physiology. By Samson Wright, M.D., M.R.C.I., Department of Physiology, Middlesex Hospital Medical School. Introduction by Swale Vincent, M.D., LL.D., D.Sc., F.R.S., Ed., Professor of Physiology, University of London. Cloth, 418 Pages. Oxford University Press, American Branch, New York, 1926.

†Lectures on Internal Medicine. By Knud Faber, M.D., Professor of Internal Medicine, University of Copenhagen, Denmark. Cloth, Pp. 14. Hoeber, New York, 1926.

estimation of therapeutic results could not be applied Louis' numerical method was the origin of medical statistics

Indeed, Morgagni had already discredited this method in saying "observations should not be counted but weighed" Diseases did not exist Only sick persons Two cases of disease were never identical A classification and calculation of probabilities based there upon were consequently impossible

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### *The Thomas Splint\**

THIS small book is an abundantly illustrated volume which will find its greatest interest in the field of orthopedic surgery, but which should also be of great value to those general practitioners who attempt to do more than first aid fracture work

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### *Intracranial Tumors and Some Errors in Their Diagnosis†*

HAPPY is the man who has reached that stage in his chosen profession where he can without fear of mockery, describe and discuss his errors as well as his successes for the benefit of those who would follow in his footsteps Sir William Osler did this to perfection in his little volume on the diagnosis of abdominal tumors Sir James Purves Stewart now does likewise with intracranial tumors The monograph is based on a review of his clinical notes on 253 patients who had signs and symptoms pointing to intracranial tumor One hundred and twenty one of these have been verified by autopsy or at operation and it is with this latter group that he deals chiefly After a brief prefatory discussion of signs of intracranial tumor he proceeds at once to his case reports which he has classified as to the location of the tumor, beginning with the frontal lobe and going backward and downward There is a chapter on multiple brain tumors, on gross errors in the diagnosis of tumors, on some pitfalls in diagnosis and finally a summary chapter on errors, avoidable and unavoidable

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\*The Thomas Splint And its Modifications in the Treatment of Fractures By N Meurice Sinclair C M G M B Ch B with a foreword by Sir Robert Jones Cloth 163 pages Oxford University Press American Branch New York 1927  
 †Intracranial Tumors and Some Errors in Their Diagnosis By Sir James Purves-Stewart K C M G C B M D F R C P 206 pages Oxford University Press American Branch New York 1927

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## EDITORIALS

### Thymus and Thyroid

AT THE present time there are many theories regarding the role of the thymus gland, some of which are fairly plausible. An endeavor is hereby made to reassemble certain experimental facts in a new hypothesis which seems to give a more workable basis for an explanation of possible functions.

The work of Alexis Carrell and others proved that in tissue culture one element is necessary to keep the cells alive and another to make growth or reproduction of cells possible. The first substance is more or less unstable, resembling somewhat in its behavior what we call complement in the blood serum, and may support an oxidation phenomenon. The second substance is cellular. Leucocytic extracts or embryonic tissue juices contain it in workable quantities. Washed suspensions of lymphocytes contain it. Thus it is not presuming too strongly to suppose that the lymphocyte, especially the common so-called "small" type, carries a growth promoting factor. This is the first premise of our hypothesis.

The next step is to show that it is possible for lymphocytes to be impregnated by the active principle of the thyroid gland before circulating generally. In adults we have the lymphocytes poured into the subclavian veins through the thoracic ducts just *before* the contents of these veins receive blood from the thyroid vein. This seems to be a rather circuitous route for the thoracic ducts unless there is some definite reason for it. May that reason not be for the purpose of impregnating the contents of the thoracic ducts with thyroïdin?

Third, if the lymphocytes carry the growth promoting factor it would seem that during the period of growth from fetal life to adolescence a state of relative lymphocytosis should exist. This we know to be a fact. During the same period, in order to meet the demand for a large supply of lymphocytes, the body could find use for a special lymphocyte producing organ. The thymus may fulfill this demand. To facilitate matters this gland pours its venous contents into the subclavian vein directly under the thyroid, while some of the thymic veins empty into the thyroid veins themselves. It looks as though nature placed the thymus where it is for the definite purpose of furnishing lymphocytes to carry the thyroid principle.

When we try to prove these premises by animal or human experimentation we encounter difficulties because nature has very wisely tucked away extra depots that can be used in emergencies. For instance, when we remove the thymus in a young animal, we often get stunted growth, but not always, because the lymph glands and spleen can function as lymphocyte producing centers. To remove every lymphocyte producing center would be impossible. The same is true in thyroidectomy. If we remove the thyroid we usually get physical types resembling cretins, though often the anterior pituitary with its colloid producing cells acts vicariously as a thyroid.

Experimental gland feeding has shown that in cases of delayed union of fractures or of old unhealed operation wounds, where the blood shows a normal lymphocyte count, good results follow the feeding of thyroid extract. This may mean that the thyroid gland has been exhausted to a point where all the thyroïdin produced is used for repair of tissues more vital than the unhealed wound and that the thyroid gland deficit can be brought to normal by thyroid feeding, thereby making it possible to impregnate all the lymphocytes with the growth promoting factor. That the lymphocytes are necessary for the proper healing and rebuilding of tissues would seem indicated by the ever-present small round cell infiltration found in traumatized or diseased areas during the stage of repair. On the other hand if we feed thyroid to patients with a leucopenia, as in cases of exophthalmic goiter, typhoid fever, influenzal bronchopneumonia, tuberculosis and certain psychoses we aggravate their distress and cause a more rapid loss of weight. Does this mean that when there are not enough lymphocytes to carry thyroïdin it becomes possible to have an uncombined thyroïdin circulating freely in the blood stream and that this results in a most serious "toxic" catabolism and upsets the balance of the other endocrines?

As far as thymus feeding is concerned it is pretty generally conceded that it has little effect. In the fresh state its nucleoproteins would probably

have the same nutritive value as pancreas, liver or kidney. Most proteins contain the phosphorous and other elements necessary for the synthesis of nucleoprotein. The craving for protein so common in the hyperthyroid states may give us a clue toward solving the riddle of improving the lymphatic organs.

—H B (P G W)

### *Pharmacologic Considerations of Acute Circulatory Collapse*

THE need for some safe, efficient, rapidly acting, and readily applicable drug to use in emergency cases in which the heart and circulation are failing has long been felt in therapeutics. Many different substances have been used in these cases. None so far known is entirely satisfactory. All have one or more failings. Nor will any combination of these drugs reach all cases.

The older drugs of the digitalis series, especially the intravenous injections of strophanthin and other preparations, all act too slowly for many cases, and these glucosides are all extremely poisonous so that the dose cannot safely be increased to bring on a rapid action. Their effects are, however, prolonged which is often an exceedingly desirable feature. While drugs like adrenalin and allied substances may act quickly on the heart and vasculature if given intravenously, these compounds usually act for only a relatively brief period, and the heart and blood vessels are very likely to pass into an even more depressed condition as soon as the action of the drug has worn off. More over ventricular fibrillation may rarely be brought on very quickly by intravascular injection of these sympathomimetic drugs. The best known example of this reaction is perhaps the development of delirium cordis under chloroform by the intravenous injection of adrenalin and as a rule drugs of this type have but little action on the heart and blood vessels if given hypodermically and still less when administered by stomach. Cardiazol and homocamfin may act when injected hypodermically. Solutions of glucose aëria, or blood transfusion must be given by intravenous injection. Tyramine and pituitrin may be given by hypodermic injection but the circulatory effects amount to but little unless these drugs are introduced directly into the blood. Normal saline may be given in various ways but the results are often more than questionable. Strychnine, camphor, caffeine, ergot and atropine now play a much smaller part as circulatory drugs than was the case a generation ago. It seems certain that their use in this direction will continue to decrease.

Practically all of these drugs have more or less widely extended actions on various other organs or structures of the body. And these extraneous actions may set a limit on their use, or on their efficiency, in acute circulatory depression. A heart that has been greatly slowed by asphyxia may often be immediately restored approximately to normal by free administration of oxygen provided the lung ventilation is ample. It seems more than probable that the introduction of some first class means of carrying on artificial respiration would promptly restore the circulation in many cases which now go on to a fatal ending. This point has been demonstrated thousands of times in the laboratory, but so far almost nothing has been done in this direction clinically.



The recent introduction of ephedrine held out a ray of hope in the line of circulatory stimulants. Varying reports as to the effectiveness, or the danger, of this drug in circulatory cases are now in the literature. The earlier pharmacologic papers, especially those of Chen<sup>1</sup> and Schmidt,<sup>2</sup> have thrown much light on the circulatory action of the drug. And a number of papers such as those by Miller,<sup>3</sup> Fetterolf and Sponsler,<sup>4</sup> Rowntree,<sup>5</sup> Jansen and Kiertman<sup>6</sup> have shown much regarding its clinical action. It seems, however, that there are a number of peculiar clinical-pharmacologic points which yet remain to be cleared up. Recent experiments carried out by Curtis<sup>7</sup> at the University of London have shown that the pressor action of ephedrine is reversed by ergotamine. Other observers<sup>8</sup> have previously noted certain differences in the degree or character of this reversal as compared with that existing between adrenalin and ergotamine.

Curtis states that three factors may be responsible for failure to obtain reversal of ephedrine by ergotamine or ergotoxine. In the first place the most striking action of ephedrine is the stimulation of the cardio accelerator ganglia and nerve endings. The rise in blood pressure following intravenous injection is due almost entirely to this action, as Chen showed that ephedrine does not produce sufficient vasoconstriction to account for the effect on the blood pressure, vasodilatation may often occur, more especially in perfusion of isolated organs and limbs. In the second place the threshold for paralysis of the accelerator nerve endings by ergotoxine is much higher than for paralysis of vasoconstrictor nerve endings, as Dale<sup>9</sup> showed in 1906. Since adrenalin produces a rise of blood pressure mainly by stimulating the vasoconstrictor nerve endings, it can readily be seen that his action is more easily reversed than that of ephedrine on the accelerator nerve endings.

The size of the doses used is important. Dale, in the paper cited above pointed out that, after a small dose of ergotoxin, small doses of adrenalin produced a fall of blood pressure, but that larger doses of adrenalin caused mixed effects. The same phenomena are also produced by ephedrine.

A recent paper by Bloedorn and Diekens<sup>10</sup> has reemphasized an element of danger which may lie in the indiscriminate use of ephedrine for conditions, such as asthma, in patients whose hearts may be especially susceptible to the action of the drug. They present the case of a patient in whom the diagnosis of asthma had been made following an acute attack of dyspnea coming on at night after a long, fatiguing trip. The patient had been given ephedrine,  $\frac{3}{8}$  gram, and took forty doses during a period of twenty days, a total of 15 grams. Following the administration of this drug, the patient developed evidence of acute cardiac decompensation accompanied by marked dyspnea, sweating, tremors, weakness, and palpitation. He developed a cardiac rate of 160 beats per minute, and the electrocardiogram revealed tachycardia of nodal origin. He exhibited pulsus alternans during this period of decompensation, and developed a large right pleural effusion. During the period that the patient was taking ephedrine, his condition grew worse, and he said that following each dose of the drug, tremor, sweating and discomfort increased. Following the discontinuance of ephedrine and the use of digitals in adequate

doses, accompanied by complete rest in bed, he showed steady improvement, the pleural effusion disappeared and he returned to a comparatively normal state in a short time. The authors conclude that ephedrine is a dangerous drug to use when patients show evidence of cardiac damage. Extreme care is necessary in the diagnosis of bronchial asthma, as not infrequently cases of so called 'cardiac asthma' are put in this group. Ephedrine may produce acute cardiac decompensation and pulsus alternans in patients with damaged hearts. If during the administration of ephedrine the patient exhibits any toxic symptoms such as palpitation, tachycardia, arrhythmia or vasomotor disturbances, the drug should be promptly discontinued. Pulsus alternans does not have the serious significance for patients who exhibit this condition with tachycardia as it has for persons with a normal cardiac rate.

Opposed to these conclusions however, it seems that certain observers have, in some instances at least obtained good results in circulatory cases by the use of ephedrine. It appears not unlikely that the condition of the intra cardiac sympathetic ganglia has much to do with the usefulness or danger of the drug's action. And it can easily be shown that by intravenous injection the drug stimulates the respiratory center in the medulla and apparently also the cardioinhibitory center. This latter action is also characteristic of digitalis. Here then we have a number of factors which are practically never thought of in clinical cases but which may be directly concerned in the action of ephedrine in any given case. And Waters<sup>11</sup> has recently noted that if ephedrine be given early, before shock has fully developed and the blood pressure has fallen to a low level in traumatic or surgical cases the chances for a favorable result from the action of ephedrine are much increased. It is to be hoped that clinicians will devote more attention to such points as these in the future.

A long experience in the laboratory has taught me that, at least in experimental animals, too much is likely to be done in these cases and that animals not infrequently die from over treatment when the chances for their recovery would have been fair if much less had been done for them. For the average case of shock with low blood pressure and with great depression of all the vital functions, I have seen best results come from the cautious intravenous injection of a moderate amount of 15 per cent glucose solution, the animal otherwise being left *entirely at rest, provided* its lung ventilation is ample.

## REFERENCES

- <sup>1</sup>Chen and Schmidt Proc Soc Exper Biol and Med 1924, **xxi**, 351
- Chen and Schmidt Chin Med Jour, 1925, **xxix**, 982
- <sup>2</sup>Miller Am Jour Med Sc, 1925 **clv**, 157
- <sup>3</sup>Fetterolf and Sponsler Arch Otol 1925, **ii**, 132
- <sup>4</sup>Rowntree Jour Pharmacol 1926, **xvii**, 261
- <sup>5</sup>Jansen and Kreitmair Klin Wehnschr 1925 **v**, 2402
- <sup>6</sup>Curtis Jour Pharmacol, 1928 **xxxiv**, 37
- <sup>7</sup>De Eds and Butt Proc Soc Exper Biol and Med 1924 **xxiv**, 800
- <sup>8</sup>Dale Jour Physiol 1906 **xxxiv**, 163
- <sup>9</sup>Bloedorn and Dickens Arch Int Med, 1928, **xlii**, 322
- <sup>11</sup>Personal communication

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The Service Bureau of the American Society of Clinical Pathologists has the following positions open for clinical pathologists

A large insurance company desires a physician for correspondence work in connection with longevity service. This position does not involve any laboratory work but requires a knowledge of clinical pathology.

Several positions are open requiring doctors with a knowledge of clinical microscopy, serology, bacteriology, physiologic chemistry, postmortem technique and tissue pathology, salary range, \$3800 to \$5000

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Any member of the Society interested in any of these positions, communicate immediately with the Secretary, Dr H J Corper, Children's Hospital, Denver, Colorado

The Registry of Technicians under the auspices of the American Society of Clinical Pathologists is being carried on in the Office of the Secretary, Children's Hospital, Denver, Colorado

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The Registry also includes an employment service, and invites physicians seeking competent technicians to communicate with this department

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## CLINICAL AND EXPERIMENTAL

### MORPHINE MEDICATION AS A METHOD OF DELAYING ASPHYXIATION\*

By FELIX P. CHILLINGWORTH MD AND ATTILIO CANZANELLI MD BOSTON

THIS investigation was undertaken in the belief that it might prove of value as a palliative measure to be used in future conditions such as existed on the submarine S-4 and thereby prolong life.

Under normal atmospheric conditions an increased need for oxygen goes hand in hand with an increased production of CO<sub>2</sub> which results in stimulation of respiration due to the small temporary excess of CO<sub>2</sub> tension in the blood. On the other hand when the subject is in an air tight compartment this same delicate mechanism hastens asphyxiation.

It is common knowledge that morphine decreases mental and physical functional activity and it was reasoned that the accompanying slowing of the respiratory apparatus would limit or retard the need for oxygen and decrease the output of CO<sub>2</sub> hence other things being equal the subject would live longer because the available oxygen would be utilized more slowly.

As this research is concerned with the reactions of the respiratory center to oxygen and carbon dioxide under mild morphine dosage, a brief review of the behavior of this center as influenced by morphine is given. Dixon<sup>1</sup> states that 'small doses of morphine depress the respiratory center in the medulla. The respiration becomes slower and the inspiration is somewhat prolonged so that it is deeper than usual the sum total of these two factors is to diminish the respiratory exchange. In a resting rabbit the air expired in thirty seconds averages about 200 cc. If 0.01 gram morphine hydrochloride is administered as an injection the average soon falls to about 90 cc, and in man a similar condition obtains, though not so exaggerated as in the example given. In

From the Department of Physiology Tufts Medical School  
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larger doses the respiration becomes very shallow, and before death, frequently assumes the Cheyne Stokes type "

Sollmann<sup>2</sup> states that "Morphine and its derivatives depress the excitability of the respiratory center. The spontaneous rhythm and the response to asphyxia and to reflexes are all lowered "

Y Henderson<sup>3</sup> points out "that very minute doses (2 mg of morphine for man) lessen the cough reflexes without producing any other noticeable change. Somewhat larger, but still nonnarcotic doses (3 to 15 milligrams of morphine) diminish the excitability for carbon dioxide, and to a lesser degree for other afferent impulses "

Higgins and Means<sup>4</sup> confirmed the fact that the threshold for  $\text{CO}_2$  is raised

Recently, Y Henderson<sup>5</sup> in discussing "oxygen want" wrote as follows "Oxygen is not a stimulant but a foodstuff. Deficiency of oxygen, beyond the first slight stimulating effect, depresses the nerve centers. In the absence of oxygen the tissues of the body cannot produce carbon dioxide. It is the carbon dioxide carried by the blood from the tissues to the brain that is the physiologic stimulant to respiration. When the center is depressed, it requires more than the normal amount of this stimulant to induce activity "

Further data are supplied by Meyer<sup>6</sup> "Under its influence the respiratory center becomes less excitable, so that the  $\text{CO}_2$  in the inspired air must be increased much more than under normal conditions in order to cause the usual increase in respiration. The sensibility of the respiratory center to reflex stimulation, such, for example, as that resulting from stimulation of the sciatic, is diminished in the same fashion as its sensibility to  $\text{CO}_2$ ."

"In man, after very small doses of morphine (3 to 10 mg) the diminished excitability of the respiratory center expresses itself by slowed and deepened breathing, for a stronger summation of the stimuli (distention of the lungs and the  $\text{CO}_2$  tension in the blood) is needed to excite the rhythmic respiratory movements "

While there is decided uniformity of opinion regarding the behavior of the respiratory center to oxygen and carbon dioxide, the reported results regarding the basal metabolic rate under morphine medication are confusing and conflicting. Dixon<sup>7</sup> states, "We have seen already that the absorption of oxygen is considerably diminished after morphine, the carbonic acid output is diminished also, although not to the same relative extent as the oxygen. As a result, the amount of carbonic acid present in the blood is usually increased slightly. Metabolism is lessened, no doubt, on account of the general quiescence. In consequence of these combined effects, less nitrogen is excreted in the urine "

In agreement with Dixon, Sollmann<sup>8</sup> writes that "The output of  $\text{CO}_2$  and to a lesser degree of urinary nitrogen, is materially decreased, mainly by the muscular quiet, in prolonged experiments also by the digestive derangement "

Nishigishi<sup>9</sup> contradicts the above authors while Boothby and Rowntree<sup>10</sup> conclude that morphine in sufficient quantities to produce sleep lowers the heat production "It should be borne in mind that the experiments

cited, pertaining to the action of morphine on the basal metabolic rate, were carried out under standard atmospheric conditions. On the other hand, subjects in air tight compartments are confronted with an atmosphere which is constantly changing, the oxygen percentage in the air of the compartment is progressively diminished whereas the CO progressively increases.

The initial effects of very small amounts of morphine are to lessen the sensibility to pain, cough, hunger, discomforts, and other disturbing influences. Before considering the use of morphine to delay asphyxia in man, tests should be made in order to secure subjects that give normal reactions to this alkaloid.

#### METHODS OF INVESTIGATION

Preliminary tests were carried out upon white rats in air tight compartments to determine dosage and differences in time of death between the morphinized animals and the controls.

The initial dose of morphine was determined by using 25 per cent of the textbook dose for white rats which is given as (minimum fatal dose, 0.40 grams per kilo or 0.40 mg/gm), i.e., 0.10 mg/gm. With this amount, rats invariably died before the controls in the same containers. Next a further reduction of 50 per cent (0.05 mg/gm) was tried with similar results.

Realizing that the dose was still too great it was again cut to 0.025 mg/gm. Even with this marked reduction the morphinized rats still died before the controls. This led us to appreciate that we were dealing with a complex depression of the respiratory center, caused by morphine, "oxygen want" plus CO action. From this point we ran our controls in separate containers of equal volume in order to secure a direct comparison, because in the same containers the normal rats were utilizing the oxygen at a more rapid rate, and this utilization naturally added to the depression of the morphinized rats and complicated the analysis of results.

We later changed from museum specimen jars to a bell jar\* with two taps, one on the top and one on the side, in order that the gas analyses (by Haldane method) could be carried out. In addition an equalizing manometer was provided which permitted the air tight jar to have atmospheric pressure at all times. This manometer bulb had a cubic capacity practically equivalent to the respiratory deficiency produced by the rats under observation and therefore this bulb volume was displaced into the bell jar toward the end of the experiment.

#### DESCRIPTION OF EXPERIMENTS AND DISCUSSION OF RESULTS

Table I gives in detail the time of asphyxiation of rats both with and without morphine. It brings out in addition several interesting facts pertaining to "oxygen want" and the corresponding reaction of the respiratory center.

Since in our experiments we have the time of asphyxiation of rats of different weights, we must interpolate this weight factor in terms of a standard weight rat. We decided to use a 150 gram rat as our standard unit throughout this investigation.

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Sealed with paraffin

TABLE I

EXP 1 2/15/28	EXP 2 2/16/28	EXP 3 2/16/28	EXP 4 2/27/28	EXP 5 2/24/28	EXP 6 2/27/28
Container Museum jar—4.5 liters	Container Museum jar—2 liters	Container Museum jars—1 liter	Container Museum jars—1 liter	Container Museum jars—1 liter	Container Museum jars—4.5 liters
Dosage 0.10 mg/gm	Dosage 0.05 mg/gm	Dosage 0.025 mg/gm	Dosage 0.025 mg/gm	Dosage 0.025 mg/gm	Dosage 0.025 mg/gm
No I Morphine Wt 130 gm	No I Morphine Wt 150 gm	No I Morphine Wt 166 gm	No I Morphine Wt 140 gm	No I Morphine Wt 167 gm	No I Morphine Wt 174 gm
No II Without Wt 175 gm	No II Without Wt 147 gm	No II Without Wt 142 gm	No II Without Wt 183 gm	No II Without Wt 165 gm	No II Without Wt 131 gm
Time 1.45 Morphine 1.50 Jar sealed 2.00 Resp 200 2.30 Resp 110 3.00 Resp 60 3.30 Died 4.25	Time 3.00 Morphine 3.04 Jar sealed 3.05 3.30 Resp 150 3.45 Resp 130 4.00 Resp 48 4.10 Died 4.14 4.20	Time 4.28 Morphine 4.29 Jars sealed 10.04 30 Resp 200 12.04 40 Resp 160 13.45 50 Resp 100 18.05 00 Resp 80 19.05 Resp 60 20.08 Resp 20 21.13 Resp 12 Rat removed and resuscitated	Time 2.53 Morphine 2.54 Jars sealed 2.55 Resp 140 12.03 15 Resp 110 11.43 29 10.83 30 36 3.33 Resp 108 3.37 Resp 80 3.40 Resp 72 3.45 Resp 40 Removed and resuscitated	Time 12.28 Morphine 12.30 Jars sealed 12.40 Resp 116 12.55 Resp 130 1.08 Resp 100 1.10 1.11 Resp 80 1.17 Resp 128 1.20 Resp 80 1.22 Resp 10 1.23 Died	Time 10.08 Morphine 10.09 Jars sealed 10.12 Resp 80 10.15 Resp 100 12.00 Resp 50 12.15 Resp 50 1.15 Resp 80 1.25 Died 1.45 2.10

This reduction of time values can be made since the time is proportional to oxygen consumption while the oxygen consumption is approximately inversely proportional to the body weight \* thus permitting direct comparisons as shown by evaluations in Table II

TABLE II

COMPARIS DURATION OF LIFE WITH MORPHINE AND WITHOUT MORPHINE IN TERMS OF 150 GRAM RAT

EXP	DOSE	WITH MORPHINE	WITHOUT MORPHINE
1	0.10	87 min	181 min
2	0.05	66 min	74 min
	0.02 <sub>1</sub>	49 min	37 min
4	0.02 <sub>1</sub>	48 min	44 min
	0.02 <sub>1</sub>	39 min	40 min
6	0.02 <sub>1</sub>	228 min	210 min

Experiment 1 was the first in which morphine was used. Two rats were placed in a four and one half liter jar. The morphinized rat lasted eighty seven minutes while the control lived one hundred eighty one minutes, here the morphine failed to delay death. This experiment indicated that we were dealing with an early death due to too great a depression of the respiratory center by morphine. It is obvious that the slowing of the use of the available oxygen caused the control a longer time.

Experiment 2 was run in the same container but its volume was reduced one half with acidulated water and the rats were supported on a floor above the water level. Here the morphinized rat lasted sixty six minutes while the control lived seventy four minutes. The dose of morphine which had been reduced 50 per cent still gave evidence of overdosage. At this point we appreciated that we were dealing with a complex depression of the respiratory center, i.e., from morphine from 'oxygen want', and from carbon dioxide. This prompted us to carry on experiments in separate airtight containers because it was evident that the rat with the raised threshold would die first.

Experiments 3, 4 and 5 are typical of the effects of morphine upon rats in separate containers (1 liter jars). Here the morphinized rats were saving oxygen for themselves alone, with the result that they outlived the controls. Incidentally, in two cases the morphinized rats were removed and resuscitated. Our usual procedure, however, was to obtain data regarding the exact extension of life with morphine. In Experiment 3 the rat with morphine lasted forty nine minutes while the control lasted thirty seven minutes. In Experiment 4 the morphinized rat lasted forty eight minutes whereas the control lived forty four minutes. In Experiment 5 the morphinized rat lasted fifty nine minutes the control forty five minutes.

Experiment 6 is a similar test, but in a 4.5 liter container. In this experiment it appears that the morphinized rat died first however reducing the time factors in terms of 150 gram rat, we find that actually the morphinized rat lasted 228 minutes while the control lasted 210 minutes.

If the rat weighed 130 grams and it lived ninety minutes its corrected duration of time would be  $\frac{10}{150}$  times 90 which equals seventy eight minutes.



Experiment 7 (Table III) is a comparison between two groups of rats. In one jar were placed three rats with morphine and one without. In the second jar were placed four rats without morphine. In this experiment all the morphinized rats lived longer than those in the second jar, but the rat without morphine in the morphine group outlived all other rats. This rat was removed and quickly returned to normal.

TABLE III

EXP 7 3/5/28	EXP 8 3/7/28	EXP 9 3/8/28	EXP 10 3/14/28
Container Museum jars—4 5 liters	Container Bell jar—10 liters	Container Bell jar—10 liters	Container Bell jar—10 liters
Dosage 0.025 mg/gm	Dosage 0.025 mg/gm	5 rats Wt 642 gm	5 rats Wt 680 gm
No I Morphine 4 rats* Wt 533 gm	No II Without 4 rats Wt 596 gm		
Time 12 51 Morphine 1 00 Jars sealed 1 42 4 dead 1 45 One dead 1 50 Three dead 1 50 Remaining rat* re- moved and resuscitated	5 rats* Wt 724 gm Time 12 52 Morphine 12 57 Jar sealed 2 00 One rat dead 2 17 Two rats dead 2 28 Three rats dead 2 30 Four rats dead Sample taken CO <sub>2</sub> 13 94 O <sub>2</sub> 4 9 4 23 Fifth rat dead	Time 2 50 Jar sealed 4 12 One rat dead 4 23 Two rats dead 4 24 Three rats dead 4 42 Four rats dead 4 47 Fifth rat dead Sample taken CO <sub>2</sub> 14 26 O <sub>2</sub> 2 87	Time 1 20 Jar sealed 2 17 One rat dead 2 45 Two rats dead 2 58 Three rats dead 3 00 Four rats dead 3 02 Five rats dead Sample taken CO <sub>2</sub> 15 49 O <sub>2</sub> 3 84

\*One rat without morphine

TABLE III—CONT'D

EXP 11 3/24/28	EXP 12 3/26/28	EXP 13 3/16/28	EXP 14 3/27/28
Container Bell jar—10 liters	Container Bell jar—10 liters	Container Bell jar—10 liters	Container Stoppered jars—15 liters
Dosage 0.025 gm/mg	5 rats Wt 1126 gm	2 rats Wt 282 gm	Dosage 0.025 gm/mg
5 rats Wt 832 gm			No I Morphine Wt 237 gm
Time 11 42 Morphine 11 46 Jar sealed 12 30 One rat dead 12 31 Two rats dead 12 55 Three rats dead 1 00 Four rats dead Sample taken CO <sub>2</sub> 11 47 O <sub>2</sub> 6 33 2 17 Five rats dead* Sample taken CO 12 34 O <sub>2</sub> 5 58	Time 11 45 Jar sealed 12 40 All rats dead Sample taken CO 14 21 O <sub>2</sub> 4 25	Time 12 28 Jar sealed 5 25 Two rats dead Sample taken CO 14 93 O <sub>2</sub> 2 58	No II Without Wt 137 gm Time 12 23 Morphine 12 34 Jar sealed 1 17 Dead Sample taken CO <sub>2</sub> 12 56 O <sub>2</sub> 6 06 1 33 Dead Sample taken CO <sub>2</sub> 13 27 O <sub>2</sub> 4 34

\*One rat without morphine

Experiments 8, 9, 10, 11, and 12 were carried out in a bell jar to permit gas analysis. Five rats were placed in the bell jar at a time. In Experiments 8 and 11, four of the five rats received morphine. Samples of the air were taken immediately after all the morphine rats had died and the final samples were taken at the end of the experiments. These experiments again show that where the morphine rats and the control rats are together, the control rats live the longest.

Experiments 9, 10, and 12 are groupings of five rats without morphine. Here samples were taken at the end of the experiments.

In Experiment 13, two rats were placed in a bell jar without morphine, samples of air being taken at the end of the experiment. In 14 we utilized a smaller jar to shorten the time of the experiments on account of the reduced number of rats.

TABLE IV  
ANALYSES OF AIR IN CONTAINER AT TIME OF DEATH OF RATS

	MORPHINE		WITHOUT MORPHINE	
	PER CENT CO	PER CENT O	PER CENT CO	PER CENT O
Exp 8	13.94	4.90	10.31	3.31
Exp 9			14.26	2.87
Exp 10			15.49	3.87
Exp 11	11.47	6.33	12.34	5.58
Exp 12			14.21	4.25
Exp 13			14.93	2.58
Exp 14	12.56	6.06	13.27	4.34
Average	12.65	5.76	14.40	3.82

Table IV gives the results of gas analyses. In the group studies rats did not die at the same time indicating that there are individual variations in their reactions to anoxemia. This was true regardless of whether they had or had not received morphine.

This series shows in all instances that the morphinized rats had a raised oxygen threshold which gives a distinct explanation for their earlier deaths when in the container with the control. It is also evident that in the death resulting at the higher oxygen level there must necessarily be a lowered CO value. The average CO and O percentage of the air in the container of the morphinized rats was 12.65 and 5.76 respectively, while the percentage of CO<sub>2</sub> and O of the control averaged 14.40 and 3.82 respectively.

The action of morphine is a depressant upon the respiratory center, and upon all activity. This general depression slows respiration in three ways: (1) it reduces the oxygen consumption; (2) at the same time the morphine raises the oxygen threshold; and also (3) raises the CO threshold. One might expect the morphinized rat to die first because its respiratory center for oxygen is set at a higher level, hence oxygen percentage below this point would be incompatible with its life, but the slowed respiration of the morphinized rat results in a slowed utilization of available oxygen, hence, the oxygen level at which death takes place is reached later than that of the control rat.

Larger dosage produces a heavy depression of the respiratory center, and the oxygen level at which death occurs is soon reached because the slowed respiratory rate does not sufficiently compensate for the raised oxygen level at which life will be maintained, i.e., the rise in oxygen level is not directly proportional to the decreased respiratory rate.

It is possible that the dosage could be further regulated so that the ratio between the decreased respiratory rate and the raised oxygen level would result in the maximum prolongation of life.

That life should be sustained in a modified way at a low percentage of oxygen (about 3 per cent) which occurs before complete asphyxiation is rather startling, yet Haldane was fairly comfortable for a short time with less than 9 per cent, and in gas-oxygen anesthesia, it is customary to allow the patient less than this amount.

All morphinized rats showed that the oxygen threshold was higher than that of the controls. This fact leads us to believe that in cases with marked anoxemia (pneumonia), it would be well to consider using other drugs to diminish the acuity of sensations and to slow respiration, or at least to prescribe morphine in reduced doses with perhaps more frequent administration.

In the above experiments the cubic capacity of the air-tight container in comparison with the volume of the rat was small. It is obvious that increase in container capacity would delay fatal apnea and extend life, the more slowly asphyxiation is produced, the less noticeable are the sensory and motor symptoms.

#### CONCLUSIONS

1 Data are presented showing that with proper dosage, morphine will prolong life in air-tight compartments.

2 Definite evidence is given that the respiratory center is depressed by a deficiency of oxygen, in spite of increasing carbon dioxide tension.

3 With marked anoxemia very small amounts of morphine affect the oxygen threshold of the respiratory center.

4 Decreased functional activity expresses a slowing of the basal metabolic rate.

#### REFERENCES

- <sup>1</sup>Dixon Manual of Pharmacology, 1925, ed 6, p 132
- <sup>2</sup>Sollmann Manual of Pharmacology, 1917, p 219
- <sup>3</sup>Henderson, Y, and Scarborough Am Jour Physiol, 1910, *xxi*, 260
- <sup>4</sup>Higgins and Meins Jour of Pharmacol, 1915, *xii*, 1
- <sup>5</sup>Henderson, Y Jour Am Med Assn, 1928, *xc*, No 8, p 586
- <sup>6</sup>Meyer and Gottheb Textbook of Pharmacology, 1914, p 338
- <sup>7</sup>Dixon Manual of Pharmacology, 1925, ed 6, p 135
- <sup>8</sup>Sollmann Manual of Pharmacology, 1917, p 227
- <sup>9</sup>Nishigishi Basal Metabolism of Morphine, Opium, and Pantoscopolumin Addicts, Jour of Oriental Med, 1926, *i*, 64
- <sup>10</sup>Boothby and Rowntree Jour Pharmacol and Exper Therap, 1923-1924, *xxii*, 99

## THE ATTITUDE OF THE PROGRESSIVE PHYSICIAN TOWARD NECROPSIES\*

BY RALPH G. MILLS † M.D., ROCHESTER, MINNESOTA

I WISH to turn back the pages of history and quote the writings of a man who was much criticized by his fellow physicians for his advanced ideas, and to make his words the basis of my remarks. A physician of Mantua, in Lombardy, named Marcellus Donatus, in 1586 wrote as follows: "Let those who interdict the opening of bodies well understand their errors. When the cause of disease is obscure in opposing the dissection of a corpse which must soon become the food of worms they do no good to the inanimate mass and they cause a grave damage to the rest of mankind for they prevent the physicians from acquiring a knowledge which may afford the means of great relief, eventually, to individuals attacked with a similar disease. No less blame is applicable to those delicate physicians who from laziness or repugnance love better to remain in the darkness of ignorance than to scrutinize, laboriously, the truth not reflecting that by such conduct they render themselves culpable toward God, toward themselves, and toward society at large."

It is the latter part of this quotation to which I would direct your attention. Scholarship alone does not necessarily imply conviction that necropsies should be conducted after every fatality. It is a fundamental weakness in our medical educational system that allows students to be graduated without being so fully convinced of the supreme value of necropsies that they would unhesitatingly grant permission for its performance on themselves or a member of their family. I am assuming, of course, that the procedure would be conducted in a satisfactory and deferential manner in keeping with the solemnity of the occasion and with the importance of the examination to all concerned.

Much of the medical knowledge available today has come directly from the necropsy table. Even a cursory survey of medical history will convince the skeptic of this fact. The Alexandrian school flourishing about 300 years before the Christian era was exceedingly active in the study of pathologic anatomy. The zeal of these physicians would put most of us to shame, but unfortunately with the fall of their civilization their medicine went with it. The Roman and later civilizations that supplanted it failed among other things to make use of the examination of dead bodies but allowed the mind and imagination full sway. The result you know only too well. The Dark Ages were dark indeed for medicine, and it was many centuries before the light began to appear.

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It is significant that the real beginnings were made by men living in the Roman States, which were later united into what we know today as Italy. A group of four men, Anthony Benivieni, then Eustachius, Rembert Dodoens, and finally Marcellus Donatus, were outstanding. It is recorded, however, that in spite of their enthusiasm and earnestness pathology did not make very rapid progress. Opposition was too strong and there were no aggressive physicians to carry on the work so energetically inaugurated.

A beginning was being made in England about this time. Francis Bacon urged that necropsies be performed for the advancement of medical knowledge. William Harvey and Glisson were of the faithful few who made practical use of necropsy material. (Glisson, too, was an ardent advocate of finding the lesions that accompany a certain train of symptoms.)

Morgagni, another Italian, nearly 200 years later, should be credited with pushing aside some of the gloom that had covered medical science for so many centuries. He it was, in 1761, who published a work, "The Seats and Causes of Diseases Investigated by Anatomy," that marks the beginning of a new era. Only since his time have necropsies become so frequent as noticeably to affect medicine.

A semblance of accurate diagnosis came into being when the performance of necropsies was more of a routine than an event. Laennec, in France, was able to interpret breath and heart sounds in terms of pathologic anatomy by means of the stethoscope which he invented, and with constant study of his patients after death. Bright made his contribution to nephritis in a similar manner. Would that there were more of his type today to complete the work so nobly begun. Semmelweis, in Austria-Hungary, was impressed by the similarity between the organs of those dead from puerperal fever and of those dead from septicemia. "Infection was his conclusion and cleanliness his remedy." Fitz, an American, discovered appendicitis at necropsy.

It is significant that practically all the men of note whose names you can recall as being outstanding in the medical practice of the past have had one thing in common. They have been clinicians who followed their fatalities to the necropsy table. The mention of the names of a few of these will suffice. Hunter and his nephew, Baille, Bichat, Graves, Corrigan, Skoda, Hodgkin, Stokes, Delafield, Janeway, Halsted and Osler. Thus has the profession profited by the experience of these masters of clinical medicine who were at the same time experts in the interpretation of pathologic conditions. Many of the great advances of the past have been made as a direct result of the intensive study of pathology as revealed at the necropsy table.

You are doubtless familiar with the biologic dictum that ontogeny recapitulates phylogeny. I have already shown that the science of medicine has been directed and moulded by the correlation of the clinical and pathologic phenomena. I wish to point out the manner in which the individual grows, and to develop the thesis that he is subject to exactly the same principles as apply to the evolution of the science.

The best diagnosticians of this and past generations have been those most careful to correlate clinical with pathologic data. The example of Osler will

be remembered in this connection, by his oft repeated remark that he never missed a necropsy in one of his fatal cases. This constituted a previous engagement, that took precedence over every other appointment no matter how important, and regardless of the prominence of the individuals concerned. Continental physicians have been more exacting in this particular than many of our own country men, hence, the rapid and phenomenal development of the leaders of medical thought in Berlin and Vienna.

The converse of this is equally true. The poorest diagnosticians of one's acquaintance are usually those who do not secure permission for the examination of their fatalities and rarely if ever attend such an examination if secured by someone else. There are unfortunately those who are not above seeking to put their more aggressive and scientific colleagues in a false position in the eyes of the relatives.

Two or three decades ago for a physician to insist on the examination of the blood, urine, sputum or feces was to expose himself to the criticism of those physicians who claimed that their diagnostic acumen was such as to make such procedures unnecessary or even absurd. Those who today take this attitude are looked upon, even by the laity as out of date and not worthy of confidence. Perhaps two decades hence those who are unable to perform necropsies or to make use of the findings in a given case will be viewed in exactly the same light. Their practice will gradually slip away from them, and when it is too late they will find that the progress of medical science has left them stranded alone, and bemoaning the cruel fate that has deprived them of patronage in their declining years. Thus the biologic law of the survival of the fittest operates in our midst.

If a physician has done his best for a patient and yet death occurs, the fault, if there be any, rests with the shortcomings of medical science and not with the individual concerned. Thus with a free conscience, the physician has a right to inquire into the reasons for the failure. Death from disease is theoretically a therapeutic failure and it is worthy of the medical idealist that he strive toward the relief of every patient entrusted to his care. The use of the necropsy is the best way to solve the problem, and to discover clues for future success.

In the same breath with which we give assent to this proposal we admit that we cannot be right in our judgment in every case. Omniscience is not a function of the human mind, as we tacitly admit when we provide lead pencils with erasers. Experience however, lessens the frequency of mistakes. In the well known principle of trial and error, we eliminate the errors by repeated trials. If the error is not discovered we continue with the trials indefinitely, as does the physician who continues to allow the undertaker to conceal his mistakes. Pursuance of this custom eventually banishes into the dim distance the sound principle of trial and error replacing it with a phantom infallibility of judgment, which is the most dangerous form of self delusion.

The symptoms of this psychosis would be interesting if the complaint were not so serious. It is insidious in onset, and is never realized by its victim. There soon ensues a feeling of self satisfaction of complacency, if not actual

euphoria One patient after another consults this physician, each is examined in the shortest possible time, is given a diagnosis, receives a prescription and is urged to call again in a few days. Patients for the most part recover, hence the logical conclusion is reached that the diagnosis and treatment were correct. Each case tends to render the fixed idea still more firmly fixed. He has no consultations, hence there is never any difference of opinion. He is always correct. He has reached a stage of diagnostic infallibility which excludes no possibility of error. A superficial general examination of the position of the body complained of, a touch on the pulse, a view of the tongue, and possibly a record of the temperature suffice for the completion of a positive diagnosis. Further examination is unnecessary and other consultations might as well be had over the telephone.

He is intensely resentful toward any physician who might consent to accept his patient in case the family demands consultation and he refuses. He considers this a personal insult, and his continued contemplation of this "unreasonable attitude" on the part of the family and "unprofessional conduct" of his associates bring him almost to the state of paranoia. His colleagues look on him as a difficult man to handle, and likely to develop intense dislikes on the least provocation. Those patients who are still loyal to him may readily admit that he is dictatorial, and they strive to avoid crossing him. This loyalty may, however, result in definite injury to the patient, or even an unnecessary fatality.

Failure to recover even when recovery was predicted, or the appearance of unlooked-for symptoms is brushed aside as of little or no importance. The possibility of there being other diseases present, or that the diagnosis is entirely wrong, is not considered. He has a routine remedy for every cough, a stock prescription for every form of headache, a specially compounded pill for pain and his own private liniment that is good for every muscle and joint disturbance. Abdominal pain is always constipation and calls for drastic catharsis. Even strangulated hernia, intussusception, and appendicitis are treated in this routine manner. An occasional fatality is an intervention of the Lord, and the physician is not in the least responsible. A "touch of pneumonia" or a "complication" may close the picture, never the rupture of a gangrenous appendix or peritonitis.

This is an extreme case, but one not so rare even in our day. There are milder cases of the same complaint, but all those so afflicted have in common one essential feature. They have no place in their life or profession for the principle of trial and error, or for the necropsy which is the only means of autocorrection. There is no known method of cure which the afflicted physician would agree to take. Prevention is the sole means of treatment of which we know. This consists of habitual direct observation of pathologic processes, and the suppression of an ultravivid clinical imagination. The remark of Francis Bacon that medical science is more professed than labored, applies only too commonly today.

You would be extremely resentful if I possessed the gift of prophesy and could tell you that certain ones here tonight would conform to this picture

twenty years hence No morphine habitue would become one if he could see the end from the beginning But I warn you that this disease will come upon even you unless you protect yourself and your professional career by the only known means of prophylaxis Remember that its onset is insidious, and that you will probably not recognize its presence when or if it does come upon you

The natural question to ask is, how can one perform necropsies if the services of a professional pathologist in the community where one expects to practice cannot be secured? I would answer you expect to make of yourself a surgeon or an internist or perhaps some other specialist You expect to work to that end until you have accomplished the desired result Do you think Addison or Bright or any of those men whose names have been mentioned hesitated for this reason? Each was his own pathologist, you can be your own When there is a will there is a way This way will be explained a little later This is merely an excuse not a reason and having had the way pointed out, persistence in this excuse should warn you that you might readily succumb to this dread disease

As a conscientious physician you have no mortal right to ask permission for that which you would not permit for yourself or a member of your own family You may not be asked to commit yourself in public, but this is a challenge to you that you cannot evade You must decide what you will do about the matter so far as your future career is concerned Putting the matter in more concrete form there are two questions which you are called on to face First, will I let it be known to my family that in the event of my death I desire that a postmortem examination shall be held, and put this so strongly that my relatives will feel it their duty to carry out my wishes if this is possible? They should seek someone to perform this examination, even if the attending physician did not of his own volition make this request Second will I oblige myself to urge an examination on a member of my immediate family, and to use my influence to secure such permission in the case of a more distant relative?

In 1914 Dr C D Spivak a Jewish physician of Denver wrote an article entitled "Postmortem Examinations Among the Jews An Historical Sketch and a Plea to Jewish Physicians" This states that for the previous ten years the Medical Advisory Board of the Jewish Consumptive Relief Society had consistently carried out the policy of seeking permission for necropsy in every fatal case in which the clinical symptoms were at all vague This was in the face of opposition of which we have little conception A society was formed to oppose this action, and to fight against this modern tendency by every known method, seeking to invoke both church and civil law This article is a strong plea to rabbis and Jewish physicians to modify the current beliefs and to nullify obsolete laws Unquestionably Spivak's influence and example have borne much fruit, at least among the more liberally minded Jews

Dr Spivak died recently, and the example that he set is worthy of permanent record which all may read who have even the slightest interest in necropsies and medical advancement In his will he directed that his body be



dissected by an equal number of Jews and Gentiles, and that thereafter his skeleton be articulated and sent to the University of Jerusalem. His wishes have been carried out. A special necropsy was arranged, preceded by ceremonies of appropriate nature, at which there were several addresses. His example has made it possible to link more closely the Medical School and the National Jewish Hospital for Consumptives. The pathologic staff of one conducts the examinations for the other. Thus does the courageous example of one man bear fruit that will benefit untold numbers.

Those who are willing to go with me so far as to agree to necropsy for themselves and for their relatives might be inclined to go one or two steps farther. If one believes in necropsies himself, he should do all he can to persuade others to do likewise. Just how this may be accomplished varies with the person and the situation in which he finds himself. There are several possibilities.

1 *General Practitioner in a Small City*—I can do no better than to call to your attention the experience of Waite in Texas. Here was a man who so thoroughly believed in necropsies that he sought until he found a satisfactory solution for his problem. He wrote two articles entitled, "Home Postgraduate Work by Means of Postmortem Examinations" and "An Experiment in Postgraduate Work at Home." In short, this experiment was the banding together of a small group of physicians for the purpose of securing as many necropsies as possible, and their detailed study. One physician recorded 100 per cent permissions in his practice, and others reported high percentages. This group reported on the results of ninety-five examinations. The cost, which was slight, was prorated, but eventually it was found advisable and worth while to employ a pathologist to assist in the work. They found it worth their energy, time and money, so would you.

2 *General Practitioner but Connected with a Hospital Staff*—You may let it be known that you are in favor of necropsies by setting a good example with the patients on your own service. Your own activity is likely to encourage others less courageous. If your hospital is to secure recognition as a suitable place for internships it is now necessary to have a pathologist connected with the staff and have at least a certain specified but ever-increasing percentage of necropsies on fatalities. This is a strong lever with which to lift the staff out of a state of lethargy and indifference.

3 *Specialist with Services in One or More Hospitals*—Your support can be given more strongly to those hospitals that meet the requirements of the American Medical Association than to those that do not. You can seek to re-educate those staff men who oppose necropsy on their patients, and strive to counteract their influence in this respect in the conduct of the hospital. By this action the hospital and all concerned will be benefited. Men who consistently oppose are likely to be less progressive and less scientific, and their places can be taken with profit by those who are capable of making much better use of the hospital facilities.

Assuming that you have done everything possible to secure permission for necropsy in every case, the problem is not entirely solved. Incidental men-

tion has already been made of the desirability of having a necropsy well performed. There are three factors involved in this phase of the question.

1 It is axiomatic that the more skillful the pathologist the more valuable will be the necropsy to all concerned. Some facts can be ascertained by any one, while others require good technique, nice discrimination and skillful interpretation. The best pathologist cannot be secured for every case, yet it is necessary to seek to improve the service in every possible way.

2 The necropsy must be performed in such a way as to assist rather than hinder the embalmer in his work. This is a technical matter with which every pathologist should be familiar or, if he is in doubt, the embalmer should be consulted. Vessels cut too short or left unligated often cause much avoidable difficulty, and lay the foundation for later opposition on the part of the undertaker.

3 An interview should be held with the relatives during which they should be fully informed of the nature of the disease, its course, the nature and reasons for any operation performed and an interpretation given of the conditions leading to the death. This should be frank and straightforward, full and explicit, yet simple and devoid of medical terms and carried out with the full consciousness of a duty faithfully performed. It is often an advantage to have someone hold this interview who has not been directly connected with the case, such as the pathologist or the superintendent, and yet one who has sufficient knowledge of details to discuss the case with ease and precision.

Special attention paid to the relatives at the time of their acute grief and a detailed explanation of the whole situation may readily dispel disturbing thoughts and correct wrong impressions with the result that the full confidence of all concerned is retained. A physician who has done his duty is entitled to retain the full confidence and respect of the relatives of a patient whom he has lost, and this he can do by facing the issue fairly and squarely in the manner suggested.

I trust that the foregoing presentation has not been without effect on you. Theoretical assent to these principles should have given place to active advocacy. Nominal agreement with these facts should have been replaced by a conviction that necropsies are essential to the progress of our science.

#### REFERENCES

- <sup>1</sup>Spirak, C. D. *Postmortem Examinations Among the Jews. An Historical Sketch and a Plea to Jewish Physicians.* New York Med Jour, 1914 xcix 1185-1189.
- Waite, W. W. *Home Postgraduate Work by Means of Postmortem Examinations.* Med Jour and Rec 1925 cxxi 210-211.
- <sup>2</sup>Waite, W. W. *An Experiment in Postgraduate Work at Home.* Texas State Jour Med 1925 xx 589-592.

# EFFECT OF STORAGE ON CHEMICAL AND ANTISEPTIC PROPERTIES OF SILVER PROTEIN SOLUTIONS\*

BY H. WALES, PH D., AND C. M. BREWER, M S., WASHINGTON, D. C.

ACCORDING to the United States Pharmacopoeia, Tenth Revision, solutions of silver protein, both mild and strong types, should be freshly prepared and preserved in well closed containers, protected from the light. A search of the literature for facts on which these specifications might be based showed such a mass of conflicting evidence that an investigation was undertaken to determine, if possible, the changes that occur in silver protein preparations during storage.

The strength of silver protein solutions was determined by three methods, the germicidal power, the inhibitory action against yeast, and potentiometric titrations of the silver ion.

## GERMICIDAL POWER

It is generally accepted that the germicidal power of argyrol and protargol is not great, but no two workers agree on the germicidal efficiency of these silver proteinates.

Derby<sup>1</sup> found that, while protargol in 2 per cent to 4 per cent solutions was effective against pneumococcus in one minute and against Staphylococcus aureus in ten minutes, a 50 per cent argyrol solution did not kill Staphylococcus aureus in two hours. Marshall and Neave<sup>2</sup> found that 0.025 per cent protargol killed Staphylococcus aureus in ten minutes. Verhoeff<sup>3</sup> reported that 12 per cent argyrol would not kill Staphylococcus aureus in one hour nor would 50 per cent argyrol in one-half hour. Kelly<sup>4</sup> found that 0.5 per cent protargol killed Staphylococcus aureus in ten minutes, while 50 per cent argyrol was ineffective in twelve hours. Post and Nicholl<sup>5</sup> found that 10 per cent argyrol killed B. typhosus in one minute but was of little value against the streptococcus, pneumococcus, or gonococcus. Martindale and Westcott<sup>6</sup> gave figures very similar to those obtained by Derby. Culver,<sup>7</sup> using the gonococcus as a test organism, found that  $\frac{1}{4}$  per cent argyrol or silvol and  $\frac{1}{8}$  per cent protargol would kill in ten minutes. Dakin and Dunham<sup>8</sup> state that Staphylococcus aureus gave growth after a six-hour exposure to 5 per cent argyrol, but that there was a diminution in the number of colonies in five minutes. Young<sup>9</sup> found that 10 per cent argyrol killed Staphylococcus aureus in urine in five minutes, while 1 per cent protargol failed to kill in one hour. Lancaster<sup>10</sup> showed that 10 per cent argyrol was capable of killing Staphylococcus aureus in one minute when only a small amount of the test culture was used in serum. Cheney<sup>11</sup> tested argyrol and protargol and obtained results from which he concluded that neither preparation was of value in the presence of pus, that

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the presence of clumps of bacteria in the test culture greatly affected the results and was responsible for the different findings, and that 1 per cent argyrol is as effective as a 25 per cent solution

This conflict in results may be ascribed to the lack of any standardized method of testing. The test organisms were grown on various media with a consequent difference in resistance. The amounts of culture used in the tests ranged from 0.5 cc to that adhering to a straight needle. The argyrol and protargol have been diluted in various ways, with distilled water, with salt solution, with serum, with hydrocele fluid, and with blood. The treated organisms have been subcultured on numerous media, including agar slants, blood agar, and broth. The age of the solutions was considered by some and not by others.

#### STABILITY OF SOLUTIONS

The results of investigations on the keeping qualities of silver protein preparations reported in the literature show a somewhat better agreement.

Bruning,<sup>12</sup> from a study of the changes in alkalinity and appearance on storage, decided that protargol solutions should be freshly prepared for use but that they might be preserved for a short period in a cool dark place. Culver<sup>7</sup> tested several silver protein preparations exposed to daylight for two months and found that the decrease in strength against gonococcus was from 50 to 75 per cent. He states that the loss in strength begins as early as the third day and is almost directly proportional to the age of the solution. Sollmann and Pilcher<sup>16</sup> tested the variations in strength of a number of silver protein solutions over a period of one year by means of the inhibitory action on the growth of yeast. They found that the strong type (protargol) decreased in strength, while the mild type (argyrol and silvol) increased. Schlee and Thiessenhusen<sup>17</sup> stored a number of preparations for periods of nine to seventeen months and determined the concentration of silver ion by means of the potentiometer. They found that during this period all solutions developed precipitates, causing a loss in the total quantity of silver in solution. The silver ion concentration decreased in some cases and increased in others. All solutions showed an increase in electrical conductivity. The total number of colloid particles suffered diminution in every case. The size of the particles increased in some cases and remained unchanged in others.

#### YEAST METHOD

The yeast method, several modifications of which are described in the literature,<sup>14, 18, 20</sup> is based on the theory that the inhibitory action of any silver protein on the growth of yeast is dependent upon the silver ion concentration and is proportional to its germicidal power. This theory has recently been questioned by Taylor,<sup>20</sup> who gives data showing that the most strongly germicidal compound he worked with had practically no inhibitory action on the growth of yeast.

Our experiments with this method have convinced us that the inhibitory action of the silver proteins on the growth of yeast cannot be compared to that produced by silver nitrate. For example, when we approach the inhibitory

concentration of silver nitrate, a slight increase in silver ion will cause a small decrease in the amount of gas produced by the yeast in a given period. On the other hand, when the concentration of silver protein is increased to give a corresponding increase in "silver ion," there is a marked decrease in the volume of gas. The data obtained over the period of study were so erratic as to be valueless for the purposes of this investigation. The results of this work will be published elsewhere.

#### POTENTIOMETRIC TITRATION OF SILVER ION

The potentiometric determination of silver salts is based on the theory that the potential developed by a silver electrode immersed in a solution of a silver salt is proportional to the concentration of silver ion. The silver ion concentration may be determined either directly from the voltage produced against standard electrodes or by titrating the solution with some ion that will reduce the concentration of silver ion. The end-point of the titration is determined by means of the enormous change of voltage shown when the last of the silver passes into the less ionized or more insoluble compound. This method does not, in fact, show the amount of the silver ion but it does show the quantity of silver more ionizable (or soluble) than the product of the reaction.

Neegaard<sup>21</sup> obtained the same values by titrating various organic silver preparations potentiometrically with chlorides, bromides, iodides, and sulphides. He found that when 3 c c of normal acetic acid were added to 100 c c of a protargol solution he was able to titrate 90 per cent of the silver instead of the 66 per cent previously obtained. He was unable to account for this, or for the ability to titrate neosilversalvarsan with sodium sulphide as the silver ion concentration of neosilversalvarsan solutions is of the same order as that of a potassium silver cyanide solution. He mentions one silver compound, hegonon, which was dissolved in potassium cyanide and the silver correctly titrated with sodium sulphide.

Treadwell, Jancett and Blumenthal<sup>22</sup> titrated silver preparations with chlorides and sulphides. They report some cases in which the chloride titrated only a portion of the silver and the sulphide titrated all. In other cases the chloride titrated all the silver.

Blumenthal<sup>23</sup> concluded that titration with sodium chloride would show ionic silver, that titration with sodium sulphide would show both ionic and complex silver, and that the total silver should be determined after oxidation with sodium peroxide.

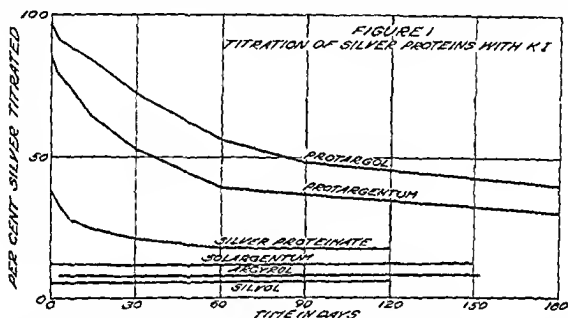
Smith and Giesy<sup>24</sup> titrated several silver preparations with standard solutions of hydrochloric acid, hydriodic acid, sodium chloride, and potassium iodide. They observed differences in the shapes of the titration curves, but all reagents gave the same end-point. In the case of the mild type, solargentum, this amounted to 11 per cent of the total silver and in the case of the strong type, protargentum, to 81.5 per cent of the silver. The curve with the mild type, using hydrochloric acid, was so irregular as to be meaningless. The authors were unable to ascribe a reason for this.

Kolthoff and Tomicek<sup>25</sup> titrated protargol containing 7.87 per cent silver

with standard solutions of sodium chloride, potassium bromide, and potassium iodide. They were unable to obtain an end point with sodium chloride. Potassium iodide gave a slightly higher figure than potassium bromide. Upon the addition of sulphuric acid before titration (4 c.c. normal acid in 50 c.c. solution), sodium chloride and sodium bromide gave approximately 7 per cent silver. Potassium iodide, on the other hand, showed one break in the titration curve equivalent to 7.08 per cent and another equivalent to 7.91 per cent silver. It is stated that the difference is due to the presence of silver chloride in the original sample. They conclude that all of the silver in protargol can be titrated with potassium iodide in the presence of sulphuric acid.

#### METHODS EMPLOYED

A 0.02 N solution of potassium iodide was chosen as the reagent for the titrations. When this was added to the silver protein solution a gradual change in voltage was produced, equilibrium being obtained quickly in most

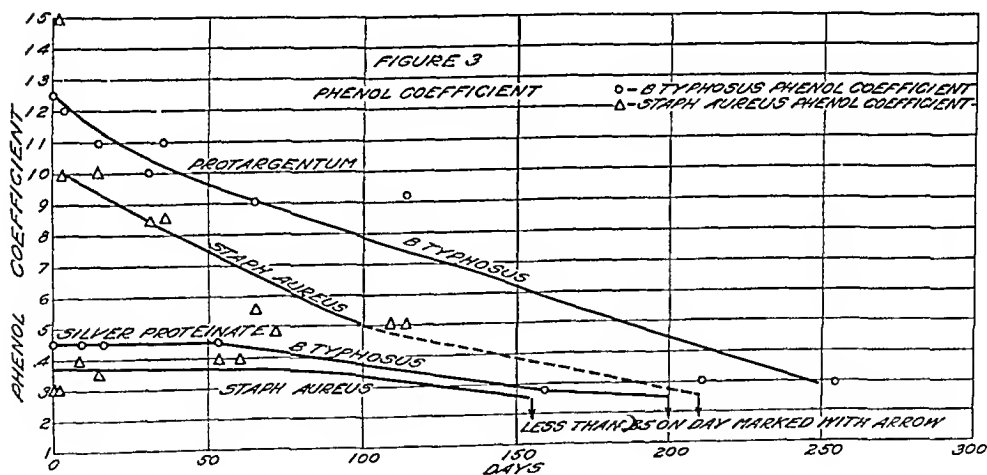
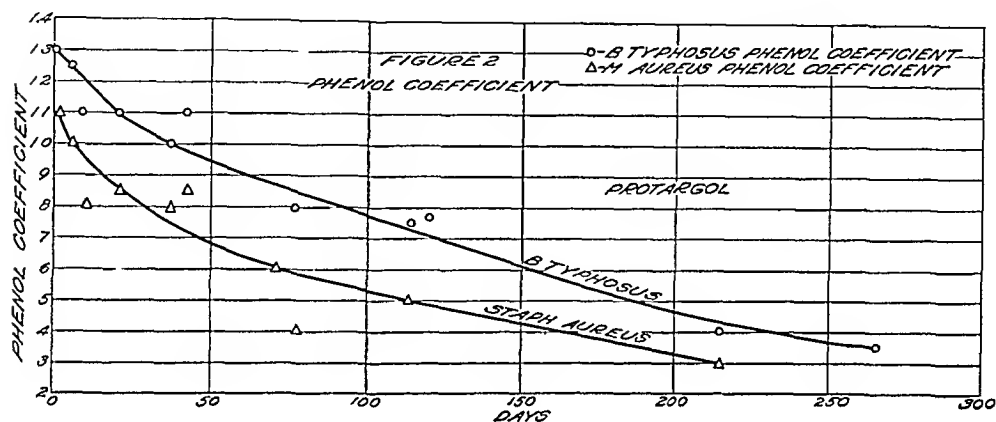


cases and a very sharp break was shown in the titration curve at the end point. The solution remained clear throughout. Experiments using the method of Kolthoff (4 c.c. normal sulphuric acid in 50 c.c. solution) were unsuccessful. It was found that each addition of potassium iodide produced an enormous change in voltage. On standing the voltage gradually returned to nearly the original value, fifteen to twenty minutes being required for it to reach equilibrium. Near the end point the product was completely precipitated as a brown mud leaving a clear colorless supernatant liquid. What appeared to be the end point gave a slightly higher value for silver ion than was obtained in neutral solutions.

Several titrations of protargol were made after the addition of acetic acid as described by Neergaard. These titrations showed about 0.1 per cent more silver than was obtained from neutral solutions.

The method followed in the bacteriologic tests is that described by G. F. Reddish<sup>2</sup> and used in the Food Drug and Insecticide Administration for testing disinfectants and antiseptics. The organisms used were *Bacillus typhosus* (Hopkins strain) and *Staphylococcus aureus*. Both are laboratory

strains whose resistance to phenol has remained constant for about five years, and comes well within the limits prescribed by the test. This method of determining the phenol coefficient was used because of its simple technic and consistent results as compared with Hygienic Laboratory and the Rideal Walker Methods. By expressing results of the bacteriologic tests in terms of the phenol coefficient the germicidal power of these solutions is compared with a standard germicide, and the phenol figure also serves as a control.



#### EXPERIMENTAL WORK

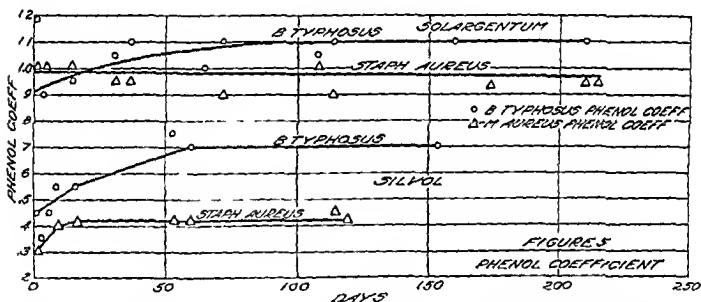
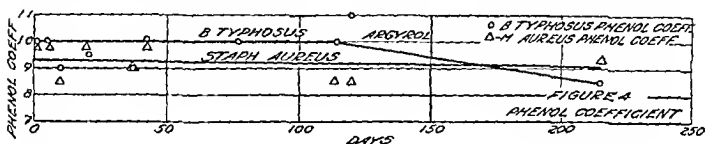
Approximately 5 per cent solutions in distilled water of six brands of silver protein, three being of the mild type and three of the strong type, were prepared. The silver contents of these solutions were determined by evaporation to dryness, igniting to destroy the organic material, dissolving in nitric acid, and titrating with standard thiocyanate. The solutions were stored in amber bottles, with cork stoppers, and at fixed times samples were tested against yeast and bacteria, and titrated with 0.02 N potassium iodide solution.

The results of the experimental work are shown graphically in Figs. 1, 2, 3, 4, and 5. The titration values for the strong silver proteins (protargol,

protargentum, and silver proteinate Mallinckrodt) decreased rapidly at a fairly uniform rate, and those for the mild type (argyrol, silvol, and solargentum) increased very slightly. The results when the yeast inhibition method was used, although rather erratic, indicated the change with age shown by the titration method. The mild type increased and the strong type decreased in strength with age.

Plotting the phenol coefficients of these solutions over a period of from five to eight months, curves are obtained which are quite similar to those representing the titration values.

As shown by the points which do not fall on the line, slight deviations were obtained from the smoothed curve. In as much as the limit of error in



determining the phenol coefficient is one or two tenths of a point, the aberrant figures may be worthy of no consideration. On the other hand, while the graphs magnify the amplitude of these deviations, the paths followed by the *Staph. aureus* and the *B. typhosus* coefficients, in at least two of the solutions, are of such close similarity as to point to a true rise and fall. Further work is in progress on this point.

The similarity between the bacteriologic curves, using either the *Staph. aureus* or the *B. typhosus* phenol coefficient, is not in agreement with the results of Taylor,<sup>9</sup> who found that the germicidal values against the two organisms did not correspond. The corresponding decreases with age of the germicidal and tritrateable potency of these solutions would seem to confirm the theory of Sollman and Pilcher that the activity is due to the silver ions, except that the relative magnitude of the germicidal powers of the strong and mild types do not correspond at all with the titration values. In fact, our results indicate that the germicidal efficiency of preparations of the mild



type of silver protein is equal to, and in old solutions higher than, that of the strong type

During the period of observation, sediment in varying quantities appeared in three of the six solutions. Accordingly, the total silver content of the solutions was redetermined at the end of the investigation. In no case was a decrease of over 2 per cent of the total silver observed. Much difficulty was experienced in all but two cases in an effort to eliminate a growth of mold, identified as *Penicillium elongatum* Baines,\* on the corks of the bottles. The mold was repeatedly cleaned from the cork, which was then washed with the silver protein solution. This was not successful, however. No explanation can be given for its absence in the two samples, one of each type, as all were kept under identical conditions.

The inhibitory action of these preparations is in marked contrast to their germicidal efficiency. The inhibitory action in this case was considered to be the absence of growth in a mixture of antiseptic and nutrient broth after five days incubation at 37° C. While the germicidal action of the freshly prepared solutions is comparable with that of phenol, their inhibitory power on the growth of *B. typhosus* was approximately ten times as great, a 1-7000 protargol solution and a 1-10,000 solution of argyrol being just sufficient to prevent growth of the *B. typhosus*. Due to the extremely slow and scanty growth of *Staphylococcus aureus* in an unfavorable medium no exact figures were obtained for this organism. The inhibiting concentrations were in approximately the same ratio but very much lower than in the case of *B. typhosus*. In this case the change with age, if any, was too slight to be measured at such high dilutions.

#### DISCUSSION OF RESULTS

The inhibitory action of silver proteins against yeast apparently cannot be used as a criterion of their strength. It is extremely doubtful whether the potentiometric titration method, or the electrical measurements of silver-ion concentration, actually furnish a measure of the silver ion. The ability to completely precipitate the preparation with iodide ion in presence of an excess of sulphuric acid tends to confirm this belief. Again, two samples of the same material purchased at different times from the same manufacturer assayed the same amount of silver, but titration with iodide showed different percentages of silver ion, assuming that silver ion is being titrated. This variation in individual products has recently been mentioned by another investigator,<sup>30</sup> who says, "It is evident that the difference between samples of the same brand is as great as, or greater than, the difference between individual brands."

Two theories have been advanced to explain the action of these preparations. One is that the strength depends on the concentration of the silver ion. Another<sup>25</sup> is that the size of the particles may have an effect on their potency. This has been shown to be true for certain other compounds.<sup>22</sup> Although there is evidence to support both theories, we believe that neither can satisfactorily

\*Identified by Dr. M. B. Church of the Microbiology Laboratory, Bureau of Chemistry & Soils.

explain the results obtained in these investigations. Gros<sup>8</sup> demonstrated that the presence of hydroxyl ions greatly increased the germicidal properties of silver preparations. Schlee and Thlessenhusen found that the size of the particles did not always change with age. It is therefore certain that the loss in silver 'ion' cannot be counter balanced in every case by a change in the size of the particle, although this offers a possible explanation for the fluctuations in potency. Finally when two such closely related substances as mercuric chloride and mercuric iodide show wide variation in their germicidal values,\* it seems hardly plausible to assume that the action of silver nitrate and that of silver proteins with widely differing protein radicals may be compared on the basis of the silver ion.

#### CONCLUSIONS

According to all methods used for testing solutions of strong silver protein deteriorate appreciably in six months while solutions of the mild type do not. Neither the yeast inhibition test nor the potentiometric titration of silver ion yields an index to the relative germicidal value. There is reason to believe that the silver ion is only partially responsible for the germicidal efficiency.

Both types of silver protein were found to have approximately the same strength in freshly prepared solutions and the concentrations ordinarily used in medical practice are germicidal.

#### REFERENCES

- <sup>1</sup>Derby Boston Med and Surg Jour 1906 xci 341 (Reprint from Trans Am Ophth Soc, ii, p 2)
- <sup>2</sup>Marshall and Neave Brit Med Jour 1906 359
- <sup>3</sup>Verhoeff Jour Am Med Assn 1906 xlv, 270
- <sup>4</sup>Kelly Brit Med Jour 1907 ii 1475
- <sup>5</sup>Post and Nicoll Jour Am Med Assn, 1910 iv 163
- <sup>6</sup>Martindale and Wescott Extra Pharmacopoeia XV
- <sup>7</sup>Culver Jour Lab and Clin Med 1918 iii 487
- <sup>8</sup>Dakin and Dunham A Handbook of Antiseptics New York 1918
- <sup>9</sup>Young White and Schwartz Jour Am Med Assn 1919, lxxiii, 1487
- <sup>10</sup>Lancaster Boston Med and Surg Jour 1920 cxxxiii, 563
- <sup>11</sup>Cheney Am Jour Ophthal 1923 vi 648
- <sup>12</sup>Bruning Pharm Zeitung 1907 lii, 156
- <sup>13</sup>Sollmann and Pilcher Jour Lab and Clin Med, 1923 viii 310
- <sup>14</sup>Sollmann and Pilcher Jour. Lab and Clin Med 1924 ix 256
- <sup>15</sup>Sollmann and Pilcher Jour. Lab and Clin Med 1924, x 38
- <sup>16</sup>Sollmann and Pilcher Jour Lab and Clin Med 1924 xi 103
- <sup>17</sup>Schlee and Thlessenhusen Ztschr Angew Chem 1924 xxxvii 837
- <sup>18</sup>Jour Assn Offic Agr Chem 1927, x 47
- <sup>19</sup>U S Pharmacopoeia X p 63
- <sup>20</sup>Taylor Jour Am Pharm Assn, 1927 xvi 820
- <sup>21</sup>Neergaard Arch f exper Path u Pharmacol 1923 c 169
- <sup>22</sup>Treadwell Janet, and Blumenthal Helv Chem Act, 1923, vi 513
- <sup>23</sup>Blumenthal Electrometrische Titrationstudien Dissertation Zurich 1924
- <sup>24</sup>Smith and Giesy Am Pharm Assn, 1925, vii 10
- <sup>25</sup>Kolthoff Jour Am Pharm Assn 1925 xiv 183
- <sup>26</sup>Kolthoff and Tomiček Rec Trav Chim 1925 xlv 103
- <sup>27</sup>Penfold and Grant Proc N S Wales 1925 lx 346 (Also 1924, 1923, 1924, and 1926)
- <sup>28</sup>Gros Munchen Med Wchnschr, 1912 lix, 405
- <sup>29</sup>Reddish Am Jour Public Health, 1927, xvii 320
- <sup>30</sup>Jour Am Med Assn, 1928, xc 840

Solutions of potassium mercuric iodide contain less mercury ion than mercuric chloride solutions of the same concentration. While the iodide is less germicidal than the bichloride towards *Staphylococcus aureus* it has five times the strength against *B. typhosus*.

# THE FAILURE OF PITUITARY SUBSTANCES TO INFLUENCE THE BASAL METABOLISM OR THE SPECIFIC DYNAMIC RE- SPONSE TO FOOD IN A NORMAL SUBJECT\*

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IN THE literature exist numerous contradictory reports of the results of feeding and injection experiments with preparations of the pituitary gland. It is generally conceded that pituitary gland preparations do not exert their optimal physiologic action when administered by mouth although Rees and Olmstead<sup>1</sup> have reported some success in the treatment of a case of diabetes insipidus when posterior lobe preparation was administered orally in salol-coated capsules. Likewise, Morris and Weiss<sup>2</sup> treated successfully a case of dyspituitarism with the administration of such extracts by mouth. Pituitrin administered orally was found by Donaldson<sup>3</sup> to be of value in cases of uterine hemorrhage. Grabfield and Prentiss<sup>4</sup> observed no influence on the nitrogen metabolism when pituitary preparations were administered orally and intramuscularly. Knaus,<sup>5</sup> however, found that pituitrin by mouth affected the uterus of a cat within eight minutes after its administration.

Other investigators have been primarily concerned with the effect of pituitary preparations on the respiratory exchange. Bernstein and Falta<sup>6</sup> observed in this connection a difference in the actions of posterior and anterior lobe preparations. In either normal individuals or patients with hypophyseal or thyroid disease, injections of posterior lobe extracts produced a moderate increase in the values for carbon dioxide output and oxygen consumption without affecting the respiratory quotient while injections of anterior lobe substance produced a lowering of the basal metabolism accompanied by an increase in respiratory quotient.

Kestner, Liebeschutz-Plaut, and Schadow<sup>7</sup> observed an increase in the specific dynamic response to foods, after oral administration of anterior lobe preparations (praphyson), while no change was evident when a mixture of posterior lobe preparations (hypophysin, physormon, and pituglandol) was fed either to normals or to cases of Frohlich's syndrome (dystrophia adiposogenitalis). These investigators could not confirm, however, the earlier observations of Liebesney<sup>8</sup> that the feeding of anterior lobe preparations may lead to a decrease in the specific dynamic response to food.

Several investigators of the German school (Plaut, et al.) have attempted to use the increase in the metabolism of an individual after a standard meal as a diagnostic test. Upon the magnitude of this response, differential diag-

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noses have been based with especial attention directed toward the hypophysis. It therefore became of interest to determine,

- 1 The influence of the administration of various pituitary preparations upon basal metabolism, and
- 2 The effect of the administration of these preparations upon the increase in metabolism following the ingestion of a standard meal

#### THE BASAL METABOLISM

Our interest in the influence of preparations of pituitary gland upon the basal metabolism was first stimulated by the response of a patient with a pituitary tumor to the administration of whole pituitary gland substance (Wilson). This patient (D R), a male, aged fifty two years, had the following symptoms weakness, somnolence, headaches right lateral strabismus, scanty beard and absence of axillary hair feminine distribution of pubic hair, unusual smoothness and softness of the skin, right optic atrophy, and facies suggestive but not typical of acromegaly. Roentgenograms showed marked enlargement of the sella turcica. His basal metabolism was -18.6 per cent (DuBois). This patient was under observation in the hospital for three months and during a part of that time received whole pituitary gland substance (Wilson) in doses approximately one gram per day (Gr IV three times a day). Table I represents his basal metabolism during the period of observation.

TABLE I

DATE	BASAL METABOLISM (DU BOIS)	PULSE RATE	REMARKS
	PER CENT		
12/23/25	-18.6	66	
12/26/25	-18.3	68	
1/4/26	-16.0	68	
1/23/26	+2.0	66	Pituitary whole gland substance 10 gram daily since 1/8
2/9/26	-8.0	78	
2/17/26	-17.0	72	Medication discontinued since 2/10
2/23/26	-23.0	68	Pituitary whole gland substance 10 gram daily starting 2/24
3/4/26	-19.0	70	
3/11/26	+3.0	110	Patient feeling poorly

These results suggest a possible effect of pituitary therapy upon the basal metabolism of this individual. The metabolism increased from -16 per cent to +2 per cent within a period of two weeks during which the patient received pituitary extract one gram daily by mouth. Seventeen days later the metabolism was -8 per cent and the pituitary extract was discontinued. Within the next thirteen days the basal metabolism had fallen to -23 per cent, a value below the initial rate determined. Pituitary extract was again administered in the same amount. Eight days later the basal metabolism was -19 per cent, a week later +3 per cent. At about this time, however, the patient began to feel poorly and developed a bronchopneumonia, dying eight days later. The pituitary tumor was confirmed at autopsy.

The fact that the second administration of pituitary substance failed to effect the basal metabolism of this individual after eight days raises a ques-

tion in regard to the cause of the increase observed following the initial administration and leaves a doubtful impression regarding the effect of the substance upon the basal metabolism

It was therefore of interest to study the effect of the administration of pituitary gland substance to a normal individual, on a constant diet who could be studied carefully over a long period of time. The subject used was a physician, twenty seven years old 182 cm tall and weighing 72 kilo. He

*SUCROSE EXPERIMENTS - CONTROLS*  
*PERCENT INCREASE METABOLISM OVER BASAL*

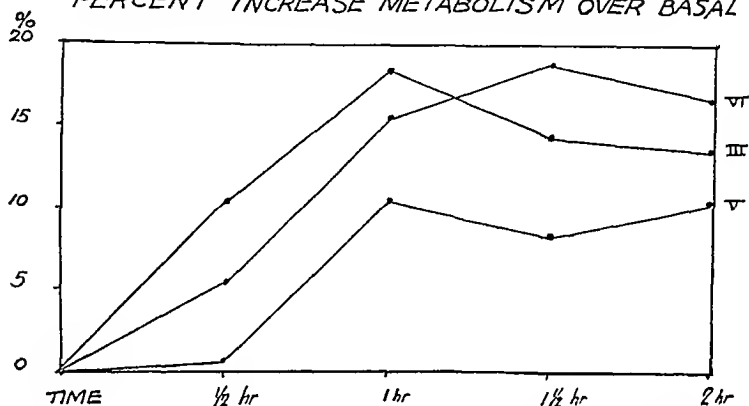


Chart 1—Sucrose 100 grams VI III V control determinations

*SUCROSE EXPERIMENTS*  
*PERCENT INCREASE METABOLISM OVER BASAL*

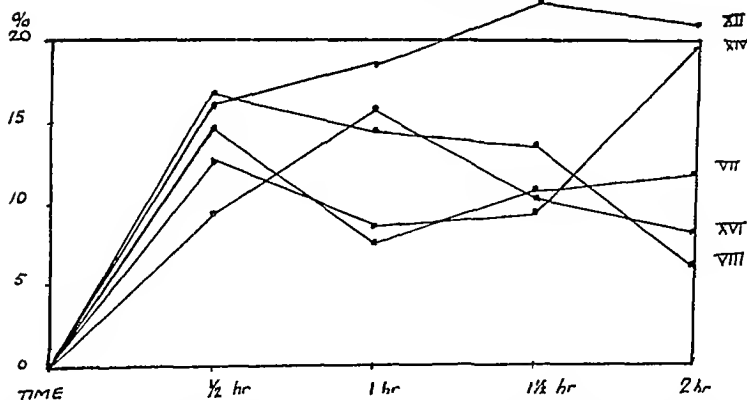


Chart 2—Sucrose 100 grams XII Antultrin intramuscularly for five days XIV Posterior lobe extract, intramuscularly for six days VII Whole pituitary gland orally for seven days XVI Posterior lobe extract intramuscularly for eleven days VIII Whole pituitary gland orally for fifteen days

was free from symptoms of any sort and worked daily as a member of the hospital staff. A physical examination at the beginning of the experiment was entirely negative. In spite of the failure of clinical symptoms of any sort, this individual was consistently found to have a low basal metabolism. Determinations were made with a Tissot spirometer at intervals during a control period of thirty days with the following results—180 per cent,

-19.0 per cent, -21.8 per cent, -18.5 per cent, -14.2 per cent, -19.0 per cent, and -20.0 per cent. These results agreed also with determinations made with the Benedict Collins Roth apparatus. In explanation of these consistently low results we can only offer the suggestion that our subject had been leading a rather sedentary life for the past eight years, and also point out the observation made by Sturges<sup>9</sup> in this connection "It has been noted that the basal metabolism in a series of normal individuals, all house officers or medical students in many instances tended toward the extreme lower border of normal that is between 10 and 16 per cent below the average. It may be that these individuals in an endeavor to cooperate attain almost complete relaxation which is similar to that observed during sleep and this might well account for the slightly diminished but not abnormal metabolism." Almeida<sup>10</sup> has done some interesting experiments demonstrating that inactivity is able to produce a lowering of the basal metabolism.

GLYCINE EXPERIMENTS  
PERCENT INCREASE METABOLISM OVER BASAL

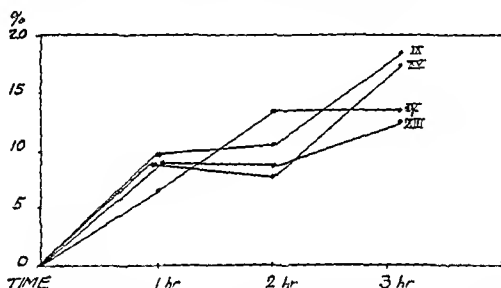


Chart —Glycine 50 grams IX Whole pituitary gland orally for sixteen days X Posterior lobe extract intramuscularly for seven days XI Control XII Antultrin intramuscularly for seven days

Since our subject presented no other abnormalities and our interest lay in the effect of pituitary substance on the basal metabolism it was decided to continue the experiment.

EXPERIMENTAL DETAILS

The Tissot method of determining the basal metabolism was employed and the gas analysis carried out by means of a carefully calibrated Hildane or Henderson apparatus. The determinations of basal metabolism were made under the usual standard conditions. The subject was placed on the following diet which contained a gram of protein per kilo: protein 72, fat 190 and carbohydrate 280 grams, calories 3118.

Whole pituitary substance (Wilson—in special coated capsules said to be resistant to the action of the gastric juice but soluble in the duodenal juice) was administered by mouth as follows: 1 gram daily for seven days.

followed by 3 grams daily for sixteen days For an anterior lobe substance antutrin (Parke-Davis) was injected intramuscularly in 4 c c amounts for seven days, while pituitary substance obstetrical (Wilson) in the same quantity was administered intramuscularly for eleven days

## RESULTS

Table II indicates the results obtained during the above periods Neither the respiratory quotient nor the basal metabolism were markedly influenced by the oral administration of whole pituitary substance over a period of sixteen days, of antutrin administered intramuscularly for seven days or by posterior lobe substance injected intramuscularly for eleven days

## THE SPECIFIC DYNAMIC RESPONSE TO SUCROSE AND GLYCINE

In addition to the determinations of the basal metabolism recorded above, a study was made of the increase in heat production following the ingestion of a standard meal and the effect of these various pituitary preparations on the magnitude of that increase The protein meals used by Plaut,<sup>11</sup> Wang, Strause, and Saunders,<sup>12</sup> Liebesney,<sup>13</sup> and others may be criticized on the ground that they contained considerable amounts of fat and carbohydrate as well as protein, and that therefore the rate of absorption may have played an important part in the time at which the greatest response was observed

In this connection it might be well to mention one experiment we performed on a patient, using a test meal similar to that described by Plaut with

TABLE II  
EFFECT OF ADMINISTRATION OF PITUITARY SUBSTANCES ON BASAL METABOLISM

EXP NO	DATE	O <sub>2</sub> PER MIN C C	CO <sub>2</sub> PER MIN C C	R.Q	CAL PER SQ M PER HR	BASAL METAB PER CENT	PULSE	REMARKS
I	11/ 9/26	215	167	0.78	32.2	-18.5	81	Control period
II	11/13/26	206	161	0.78	30.9	-21.8	70	Control period
III	11/18/26	215	168	0.78	32.2	-18.5	81	Control period
IV	11/24/26	214	164	0.77	32.0	-19.0	75	Control period
V	11/27/26	227.5	170	0.75	33.9	-14.2	72	Control period
VI	11/30/26	208	167	0.80	31.4	-20.5	74	Control period
VII	12/ 7/26	214	170	0.80	32.2	-18.5	75	Whole pituitary gland 1 gm daily since 12/2
VIII	12/22/26	223	174	0.78	33.4	-15.4	72	Whole pituitary gland 3 gm daily since 12/7
IX	12/23/26	214	168	0.78	32.1	-18.7	73	Whole pituitary gland 3 gm daily since 12/7
X	1/11/27	205	169	0.82	30.6	-22.5	71	Control No pituitary since 12/23/26
XI	1/18/27	211	166	0.79	31.1	-21.3	73	Control No pituitary since 12/23/26
XII	1/23/27	202	159	0.78	29.9	-24.3	73	Antutrin 4 c c daily since 1/18
XIII	1/25/27	208	164	0.79	30.9	-21.8	70	Antutrin 4 c c daily since 1/18
XIV	2/ 3/27	228	168	0.74	33.3	-15.7	73	Posterior lobe extract 4 c c daily since 1/25
XV	2/ 4/27	206	163	0.79	30.5	-22.8	70	Posterior lobe extract 4 c c daily since 1/25
XVI	2/ 8/27	218	174	0.80	32.4	-18.0	80	Posterior lobe extract 4 c c daily since 1/25

TABLE III  
THE EFFECT OF THE INGESTION OF SUCROSE AND GLYCINE

TABLE III

THE EFFECT OF THE INGESTION OF SUCROSE AND GLYCINE

EXP NO	BASAL METAB PER CENT	METABOLISM AFTER INGESTION OF SUCROSE	REMARKS							
		1/4 HOUR	RESP QUOT	1 HOUR	RESP QUOT	1 1/2 HOUR	RESP QUOT	2 HOURS	RESP QUOT	
		PER CENT INCREASE OVER B M		PER CENT INCREASE OVER B M		PER CENT INCREASE OVER B M		PER CENT INCREASE OVER B M		
III	-18.5	10.4	0.92	18.2	0.94	15.2	0.93	13.7	0.93	Control
V	-14.2	0.2	0.91	10.7	0.90	8.9	0.86	11.2	0.93	Control
VI	-20.5	5.1	0.92	15.4	0.92	19.4	0.91	16.1	0.93	Control
VII	-18.5	15.0	0.96	7.6	0.90	10.6	0.91	11.9	0.91	Whole pituitary gland 1 gm for 7 days
VIII	-15.4	16.7	0.95	14.0	0.96	13.4	0.82	7.1	0.89	Whole pituitary gland 1 gm for 15 days
XII	-24.3	16.2	0.85	18.5	0.89	22.3	0.93	21.0	0.92	Antutrin 4 cc intramuscularly for 5 days
XIV	-15.7	12.7	0.89	8.1	0.88	9.9	0.95	19.2	0.90	Posterior lobe 4 cc intra muscularly 0 days
XVI	-18.0	9.1	0.95	15.5	0.95	10.1	0.90	8.1	0.93	Posterior lobe 4 cc intra muscularly 11 days

TABLE IV

TABLE IV  
THE EFFECT OF THE INGESTION OF SUCROSE AND GLYCINE

THE EFFECT OF THE INGESTION OF SUCROSE AND GLYCINE													
METABOLISM AFTER INGESTION OF GLYCINE													
EXP NO	BASAL METAB PER CENT	RESP QUOT	1 HOUR				2 HOURS				3 HOURS		REMARKS
			PER CENT INCREASE OVER D M		RESP QUOT	PER CENT INCREASE OVER B M		RESP QUOT	PER CENT INCREASE OVER D M		RESP QUOT		
IV	-19.0	0.77	6.8	0.81		13.9	0.88		13.4	0.81		Control Whole pituitary glands 3 gm daily 16 days Antutrin 7 days Posterior lobe 7 days	
IX	-18.7	0.78	9.8	0.82		10.3	0.79		18.7	0.83			
VIII	-21.8	0.79	9.8	0.84		8.9	0.88		11.7	0.87			
XV	-22.8	0.79	9.4	0.86		7.9	0.88		17.1	0.84			



the exception that a small amount of barium sulphate was incorporated with the chopped meat. It is of interest that at the end of three hours there was still visible by means of the fluoroscope a bolus of barium-containing food, this in a patient without recognizable gastrointestinal abnormality. Despite the fact that the stomach still contained food, there was an increase in the heat production at one and two hours after ingestion. To us this factor of absorption is a large and uncertain one and must be carefully considered in all experiments dealing with the ingestion of materials by the gastrointestinal tract. Nor do we feel that the simpler substances we have used clears up this difficulty entirely, but that these substances would seem to be more readily and quickly absorbed hardly seems unreasonable.

To obviate this difficulty as much as possible, simple substances, sucrose and glycine were used in this study. Only four experiments were carried out with glycine since it was very unpleasant to ingest and fairly expensive. The amount of glycine used was in each case 50 grams and of sucrose 100 grams. To reduce the nausea which may be produced with glycine, it was administered in a large volume of fluid, 750 cc, while with sucrose only 225 cc were used. The results of these experiments are summarized in Tables III and IV.

#### DISCUSSION

Although consistent results were obtained for determinations of basal metabolism throughout, in our control experiments in which the metabolism following the ingestion of sucrose was determined at periods of one half, one hour, one and one-half and two hours, following its administration, we were unable to obtain consistent results with respect to either the magnitude or time of the maximal increase in heat production. The type of curves which we obtained are similar to those obtained by Benedict<sup>11</sup> but disappointing when compared to the consistent results which Lusk<sup>15</sup> obtained following the administration of glycine to dogs. With glycine we obtained only one control experiment.

Following the administration of the various pituitary preparations, the same irregularity of response to ingested sucrose was seen as was observed in the control periods. The response to glycine throughout was more consistent, and varied but little in the periods after pituitary substance was administered from the response in the control period.

The failure to demonstrate a regular quantitative metabolic response to the administration of these two relatively simple substances to a subject under ideal conditions casts doubts on the diagnostic value of the specific dynamic response to a protein meal as advocated by Plant<sup>12</sup> and Liebesnev<sup>13</sup>. Lauter<sup>14</sup> found a marked irregularity in the response of the metabolism of individuals who were fed a test meal of 200 grams of beefsteak and 50 grams of bread and decided that the conclusions drawn from single three hour, or shorter determinations of specific dynamic action as used by Plant, and also Liebesnev cannot be considered as based on adequate evidence.

As far as can be ascertained from our data the administration of various pituitary substances influenced little the irregularity noted above.

It would seem therefore that before such a determination is available for diagnostic purposes, it is necessary to find a substance if possible which when administered will result in a definite even response of the metabolism of an individual and of a group of individuals

#### SUMMARY AND CONCLUSIONS

1 Administration by mouth of whole pituitary substance (Wilson) and by intramuscular injection of antuitrin (Parle Davis) and of pituitary solution obstetrical (Wilson) over comparatively long periods of time failed to influence the basal metabolism or the respiratory quotient in an apparently normal subject

2 The increases in the metabolism after the administration of sucrose and glycine separately were so irregular both with regard to time and magnitude of the maximal response both during the control periods and also following the administration of the above substances that conclusions regarding the effect of the administrations of pituitary substances upon the specific dynamic effect of food are unwarranted

The authors wish to thank Prof L H Newburgh in whose laboratory this work was carried out for his suggestions and interest in the problem Dr Franklin Johnston for assistance with the technical procedures and many other kindnesses Miss Marie O Connor for those determinations of basal metabolism done by the Benedict Collins Roth Method and Miss E Ackerman for the preparation of the diet

#### REFERENCES

- <sup>1</sup>Rees, M H, and Olmstead W H The Use of Pituitary Extracts by Mouth in Treatment of Diabetes Insipidus *Endocrinology* 1922 vi 230
- <sup>2</sup>Rees M H Action of Pituitary Extracts When Introduced Into the Alimentary Canal *Am Jour Physiol* 1922 lxvii 1922
- <sup>3</sup>Donaldson *Proc Royl Soc Med (Therap Section)* 1921 17 19
- <sup>4</sup>Morris R S, and Weiss H B Dyspituitarism *Jour Am Med Assn* 1922 lxxviii 1522
- <sup>5</sup>Grabfield G P, and Prentiss A M Effect of Pituitary Preparations on the Nitrogen Metabolism *Endocrinology* 1925 ix 144
- <sup>6</sup>Knaus H H Action of Pituitary Extract Administration by Mouth, *Brit Med Jour* 1926 i 234
- <sup>7</sup>Bernstein and Falta *Kongr f Inn Med Verh* 1912, 36 *Deutsch Arch f klin Med* 1918, 125
- <sup>8</sup>Kestner O, Liebeschütz Plaut R and Schadow H Spezifisch Dynamische Wirkung Hypophysenvorderlappen und Fettsucht *Klin Wchnschr* 1926, xxxvi, 1647
- <sup>9</sup>Liebesney, P Hypophysis and Metabolism *Wien klin Wchnschr* 1925, xxxviii, 780  
Influence of the Hypophysis on the Metabolism *Wien klin Wchnschr* 1925, xxxviii 780 *Verh der 4 Tagung fer Verdauungs und Stoffwechsellkrankheiten in Berlin* 1925 186 *Biochem Ztschr* 1924 cxlv 308
- <sup>10</sup>Sturgis C C Myxedema *Med Clin North America* 1920 v
- <sup>11</sup>de Almeida A O *Jour de Physiol et de Pnth Generale* 1920 xviii 712 908
- <sup>12</sup>Plant R Gaswechseluntersuchung bei Fettsucht und Hypophysiskrankungen *Deutsch Arch fur Klin Med* 139 149 285 1922 *Deutsch Arch f klin Med*, 1922 23, cxli cxlii 266 286
- <sup>13</sup>Wang O C Strouse, S and Saunders A Studies on the Metabolism of Obesity III The Specific Dynamic Action of Food *Arch Int Med* 1924, xxxv, 573
- <sup>14</sup>Liebesney P Cited above
- <sup>15</sup>Benedict and Carpenter *Carnegie Institution of Washington Publication* 1918, 261
- <sup>16</sup>Lusk G Specific Dynamic Action of Various Food Factors *Medicine* 1922 i 311
- <sup>17</sup>Lauter S Zur Genese der Fettsucht *Deutsch Arch f klin Med* 149 100

# A STUDY OF THE OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION OF GENTIAN-VIOLET AND ACRIFLAVINE SOLUTIONS WITH REFERENCE TO THEIR STABILITY AND THERAPEUTIC VALUE\*†

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THE intravenous use of gentian-violet and neutral acriflavine has given exceptionally variable and conflicting results in clinical and experimental work in recent years. Many of the factors supposedly contributory to these variations have been studied by various investigators, but the exact causes have never been satisfactorily explained. A fairly comprehensive view of the different phases on the subject may be obtained from papers of Klügler,<sup>10</sup> Brill and Meyers,<sup>1</sup> Young and Hill,<sup>14</sup> Reed and Lum,<sup>12</sup> Graham-Smith,<sup>7</sup> Burke and Newton,<sup>4</sup> Burke and Rodier,<sup>5</sup> Meleney and Zau,<sup>11</sup> Spencer,<sup>13</sup> and needs no elaboration here. Considerable attention has been given in these studies to the reaction of the dye solutions, to their toxic effect on body cells, to their bactericidal and bacteriostatic action in the presence of body fluids, and to their solubility in various solvents, but scarcely any work has been done on the osmotic pressure of these solutions.

A cursory examination of the various solvents recommended for the preparation of these dyes for intravenous injection suggests that there must be wide variations in the osmotic pressure of the solutions. Since the danger attending the use of hypotonic and hypertonic solutions for intravenous injections is generally recognized, it is possible that these variations affect the final results. The purpose of this study was to determine the osmotic pressure of some of the recommended solvents and dye solutions, and also to prepare solvents and dye solutions that are isotonic with the blood and have approximately the same reaction as the blood, thus making it possible to study their therapeutic value under better standardized and more carefully controlled conditions.

## THE DETERMINATION OF OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION OF DYE SOLVENTS AND DYE SOLUTIONS

Several of the solvents generally recommended for the preparation of gentian-violet and neutral acriflavine and several of the recommended solutions of these dyes were prepared for osmotic pressure and  $P_H$  determinations. The salts used for the solvents throughout these studies were Baker's c p chemicals. As these contain perceptible amounts of impurities, the osmotic pressure and  $P_H$  values naturally vary somewhat from values that would be

\*From the Division of Bacteriology Washington Agricultural Experiment Station

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obtained from absolutely pure chemical compounds. The dyes were the gentian violet (medicinal) and the neutral acriflavine (for injections) prepared by the National Aniline and Chemical Company, of New York. It was found that different lots of these dyes varied somewhat in  $P_H$  but did not vary perceptibly in osmotic pressure. The dyes used in all the experiments here described were from the same lot. The osmotic pressure and  $P_H$  determinations of the dye solutions were always made on fresh solutions which were prepared at a temperature of 40° to 45° C. It was observed that with certain solvents temperatures higher than 45° C. had a marked effect on the stability of the dye solutions. Osmotic pressure determinations were made by the indirect freezing point and Harris and Gortner's<sup>9</sup> formula to calculate the osmotic pressure in atmospheres. This formula is  $\pi = 12.06\Delta - 0.021\Delta^2$  in which  $\pi$  is the osmotic pressure in atmospheres and  $\Delta$  is the lowering of the freezing point in degrees centigrade. The  $P_H$  determinations were made by the potentiometer method using the hydrogen electrode.

The results of the determinations of the series of solvents and solutions are given in Table I. They show that the osmotic pressure varies from less than 1 atmosphere in dye solutions prepared with distilled water to more than 12 atmospheres in dye solutions prepared with 3 per cent sodium bicarbonate. The former is greatly hypotonic and the latter highly hypertonic with human blood which has an osmotic pressure of approximately 6.7 atmospheres. These wide variations may influence the results obtained by the intravenous use of these solutions if they are introduced into the blood stream in considerable amounts. Hanzlik, et al.,<sup>5</sup> observed distinct symptomatic changes following the intravenous injection of 0.1 per cent hypertonic sodium chloride which were not noticeable following injections of 0.9 per cent isotonic sodium chloride. On the other hand 50 per cent glucose and 3 per cent sodium bicarbonate solutions both of which are greatly hypertonic have been used intravenously without serious effects. This suggests that the nature of the substances as well as the osmotic pressure of the solution may be important in bringing about symptomatic changes in the blood.

The  $P_H$  of these various solvents and dye solutions also show marked differences. There is considerable experimental work claiming that the hy

TABLE I  
OSMOTIC PRESSURE AND HYDROGEN ION CONCENTRATION OF SEVERAL SOLVENTS  
AND DYE SOLUTIONS

SOLVENTS	SOLVENT		AFTER ADDITION OF 1 PER CENT GENTIAN VIOLET		AFTER ADDITION OF 1 PER CENT NEUTRAL ACRIFLAVINE	
	$P_H$	P	$P_H$	P	$P_H$	P
Distilled water	6.8		5.24	0.54	6.76	0.93
0.85 per cent sodium chloride	7.23	6.09	5.41	6.62	6.65	6.62
3 per cent sodium bicarbonate†	8.72	12.18	8.33	2.30	8.12	14.72
Buffered distilled water (0.300 gm $KH_2PO_4$ + 0.387 gm $K_2HPO_4$ per 100 c.c.)	6.67	5.32	6.13	2.34	6.45	3.06
4 per cent glucose	6.91			5.40		5.72

Osmotic pressure in atmospheres

†Solution precipitated when dye was added to the solvent

hydrogen-ion concentration of dye solutions is a potent factor in toxicity and bactericidal effect of the dye. This is especially emphasized in the studies of Browning et al.,<sup>2</sup> Eggeith,<sup>1</sup> and Burke and Newton.<sup>4</sup> Thus both osmotic pressure and reaction may be important factors in intravenous medication. Since these factors are controllable, it would seem desirable to regulate them to closely approximate those of the blood. The following experiment was designed with that object in view.

THE PREPARATION OF GENTIAN-VIOLET AND NEUTRAL ACRIFLAVINE SOLUTIONS WITH APPROXIMATELY THE SAME OSMOTIC PRESSURE AND HYDROGEN-ION CONCENTRATION AS THAT OF THE BLOOD

In selecting solvents for dyes for intravenous injections it is important to use materials that are not injurious when injected into the blood stream. Furthermore, these materials should have the property of increasing the  $P_H$  of the dye solutions as most dyes used for intravenous injections are acid in reaction. With the possible exception of sodium bicarbonate, Hanzlik et al.<sup>5</sup> found disodium phosphate to answer this purpose most satisfactorily. They injected a slightly hypertonic (1.1 per cent) solution and a distinctly hypotonic (5 per cent) solution into dogs with resulting beneficial changes in their blood. Since sodium bicarbonate is not suitable as a solvent for gentian-violet and neutral acriflavine for reasons explained below, the phosphate salts were selected. For convenience the tri-, di-, and monopotassium phosphates were used in proportions suitable to obtain the desired osmotic pressure and  $P_H$ . These salts are well-known for their buffer effect and should prove very effective in correcting the acidity of the dyes. This was true with neutral acriflavine but was not so easily accomplished with gentian-violet. It was found that gentian-violet also has a powerful buffer effect which makes any change in its reaction to the alkaline side somewhat difficult, especially when the point of neutrality is approached. The reaction of  $P_H$  7.6 was found to be about the critical point when potassium phosphates were used as solvents. At any  $P_H$  above this point the gentian-violet solution became unstable and this resulted in a general breaking down of the solution, making it unreliable for experimental work. Sodium bicarbonate seemed to have a similar effect. When 1 per cent gentian-violet was freshly added to a 3 per cent solution of sodium bicarbonate and the  $P_H$  determined immediately the potentiometer readings constantly changed until a  $P_H$  of above 8 was reached. This usually required several hours and resulted in a general breaking down and precipitation of the solution, making it unfit for the purpose of this study.

The use of an isotonic solution of dipotassium phosphate alone did not correct the reaction of gentian-violet in 1 per cent concentration to the desired  $P_H$ , so it was necessary to make use of tripotassium phosphate which has a much greater alkaline reaction. By making repeated trials with various proportions of tripotassium phosphate and dipotassium phosphate a 1 per cent gentian violet solution with an osmotic pressure of 6.48 atmospheres and a  $P_H$  of 7.64 was finally obtained. The solvent consisted of a mixture of 0.050 mols tripotassium phosphate and 0.050 mols dipotassium phosphate dissolved per

liter of freshly distilled water. When 1 per cent gentian violet was added to this at 40° C the resulting solution was relatively stable for twenty four hours as shown by repeated osmotic pressure and  $P_H$  determinations during that time. Another satisfactory solvent, consisting of a mixture of 0.051 mols tripotassium phosphates and 0.052 mols sodium acetate per liter of distilled water, was obtained. This also made a stable isotonic, all aine solution with 1 per cent gentian violet.

As noted above the preparation of a 1 per cent isotonic neutral acriflavine solution with the desired  $P_H$  did not present any difficulties because the buffer action of neutral acriflavine did not interfere to any marked extent. A few of the various combinations of the salts used as solvents and their effects on osmotic pressure and  $P_H$  of the dye solutions are reported in Table II. Molar concentrations of the salts rather than percentages by weight are given for convenience.

The results show how easily the  $P_H$  of gentian violet solutions is affected by small variations in the amount of buffer salts at the critical point of reaction for gentian violet. This should serve to emphasize the necessity of observing careful precision in preparing solvents for dye solutions for intravenous medication. The experiment demonstrated the possibility of preparing 1 per cent gentian violet or neutral acriflavine solutions that have the same osmotic pressure as the blood and have a  $P_H$  similar to that of the blood.

It seemed now desirable to determine the effect of these standardized solutions on the therapeutic value of these dyes. The following experiments were undertaken for that purpose.

#### ANIMAL EXPERIMENTS WITH GENTIAN VIOLET

The fact that gentian violet is distinctly more acid than neutral acriflavine and consequently is more apt to be administered intravenously as an acid solution would seem to indicate that the changes if any resulting from the standardization of osmotic pressure and  $P_H$  could best be demonstrated by using this dye. To demonstrate these changes it was decided to use large

TABLE II

OSMOTIC PRESSURE AND HYDROGEN ION CONCENTRATION OF VARIOUS BUFFERED SOLVENTS AND BUFFERED DYE SOLUTIONS

MOLAR CONCENTRATION OF BUFFERS	SOLVENTS	AFTER ADDITION OF 1 PER CENT GENTIAN VIOLET		AFTER ADDITION OF 1 PER CENT NEUTRAL ACRIFLAVINE	
		$P_H$	$P$	$P_H$	$P$
0.06, M $K_2PO_4$ + 0.030 M $K_2HPO_4$		10.37	6.37	8.83	0.78
0.059 M $K_2PO_4$ + 0.030 M $K_2HPO_4$		10.34	6.04	8.68	6.37
0.050 M $K_2PO_4$ + 0.050 M $K_2HPO_4$		10.23	6.31	7.64	6.48
0.048 M $K_2PO_4$ + 0.052 M $K_2HPO_4$		10.18	6.19	6.51	6.61
0.054 M $K_2PO_4$ + 0.046 M $NaCHO_3$		10.30	6.07	8.82	0.49
0.051 M $K_2PO_4$ + 0.052 M $NaCHO_3$		10.21	6.13	7.52	6.44
0.040 M $K_2PO_4$ + 0.070 M $K_2HPO_4$		8.81	6.20		
0.12 M $K_2HPO_4$ + 0.02 M $K_2HPO_4$		7.29	6.18		
				8.63	7.19
				7.10	6.98

Gram mols per liter

† Osmotic pressure in atmospheres

doses for intravenous injections Since gentian-violet is known to possess greater bactericidal properties in alkaline solutions than in acid solutions, the  $P_H$  of the dye was adjusted slightly above that of the blood The solvent consisted of a mixture of 0.050 mols tripotassium phosphate and 0.050 mols dipotassium phosphate dissolved in a liter of freshly distilled water One per cent gentian-violet was added to a convenient amount of this solvent at a temperature of  $40^{\circ} C$ , the solution was gently shaken for two or three minutes, and filtered immediately through filter paper By making corrections for the dye remaining in the flask and in the filter, it was found that the filtered solution contained 0.86 per cent gentian-violet This solution was made up fresh for each injection and was injected at body temperature in the marginal ear vein of rabbits The osmotic pressure of the gentian-violet solution was 6.48 atmospheres and the  $P_H$  was 7.64

*Experiment 1—To determine the effect of the intravenous injections of large doses of gentian-violet on normal rabbits*

Two full grown, normal rabbits weighing between 3000 and 4000 gm and one half grown rabbit weighing 1940 gm received each 20 mg per kilo of isotonic alkaline gentian violet solution The injections were made in the marginal ear vein very slowly, allowing fifteen to twenty minutes for each injection One of the full grown rabbits developed spasms a few minutes after injection and died The other two did not seem to be affected and resumed eating grass as soon as they were returned to their cages One of these was later used for further experimentation on dye tolerance

The experiment demonstrated that 20 mg per kilo of the isotonic alkaline gentian violet was not unusually toxic as two out of three rabbits did not show any injurious effects This gave reasonable assurance that the toxicity of the dye was not increased by the use of the isotonic alkaline solution and that large doses could be used in determining its therapeutic value This was done in the following experiment

*Experiment 2—To determine whether a large dose of isotonic alkaline gentian violet in the blood of a rabbit is sufficient to check an infection with Staphylococcus aureus*

Two full grown, normal rabbits were injected intravenously with 2 cc of a heavy suspension of a virulent twenty four hour culture of Staphylococcus aureus Twenty four hours later both animals showed severe symptoms to the same degree At this time one of the rabbits received 17 mg per kilo of isotonic, alkaline gentian violet solution This was given very slowly allowing twenty minutes for the injection No symptoms followed the injection There was some improvement four hours after the injection The following day the control rabbit died while the treated animal showed marked improvement as it was breathing normally and taking food The following two days the animal was eating normally but was losing weight rapidly The rabbit died thirteen days after the injection of the dye An autopsy showed typical lesions in the heart, kidneys, and liver The experiment showed that 17 mg per kilo of isotonic alkaline gentian violet retarded death resulting from a Staphylococcus aureus infection but did not prevent death

The experiment was repeated with two half grown, normal rabbits Instead of injecting 17 mg per kilo of isotonic alkaline gentian violet, 15 mg per kilo were administered, allowing twenty minutes for injection No violent symptoms developed following the injections, but the animal appeared listless and died three hours later The control rabbit did not die until the third day after the injection with a 2 cc suspension of Staphylococcus aureus Since it was possible that the young rabbit receiving the dye happened to be more susceptible than the average rabbit, the experiment was repeated once more

Two half grown, normal rabbits were injected with a 2 cc suspension of virulent Staphylococcus aureus and twenty four hours later one received 15 mg per kilo of dye very slowly as in the previous experiment No violent symptoms appeared, but the rabbit did

not show any signs of improvement six hours later. Death followed eighteen hours after the administration of the dye. The control rabbit died the night following the third day after the injection with *Staphylococcus aureus*.

The results of these experiments showed that the half grown rabbits with severe symptoms of *Staphylococcus aureus* infections were unable to tolerate 15 mg per kilo of the isotonic, alkaline dye solution and suggested that the toxicity of the dye was not reduced materially by controlling its osmotic pressure and  $P_H$ . To test this point more definitely the following experiment was designed.

*Experiment 3—To determine the toxic effect of repeated injections of 15 mg dye per kilo on a normal rabbit*

In Experiment 1 the rabbit that received 20 mg of dye per kilo and showed no symptoms following the injection was kept for three months and selected for this experiment because the animal had proved to be tolerant to an excessive dose of isotonic, alkaline gentian violet. At the end of three months the rabbit was still in perfectly normal condition and was now given 15 mg per kilo of the isotonic alkaline gentian violet in the usual manner. When approximately 10 mg of the dye were injected the rabbit showed signs of depression and the injection was discontinued immediately. Spasms developed suddenly and the animal died. Although the results of this single experiment are insufficient to draw definite conclusions, they seem to indicate that the tolerance of rabbits for gentian violet is a function that is not constant for the individual but one that may vary at different periods, although no differences in the physical appearance of the individual may be noticeable.

#### DISCUSSION

Many factors have been discussed in the literature in an attempt to explain the causes responsible for the conflicting results so generally obtained in intravenous dye therapy. Perhaps the hydrogen ion concentration has received more attention than any single factor. Burke and Grieve<sup>3</sup> believed that the ultimate fate of the dyes as chemotherapeutic agents in injections is largely dependent upon the effect and control of the hydrogen ion concentration. The results of the experiments reported here do not support this belief. The beneficial effects of gentian violet solutions whose osmotic pressure was isotonic with the blood and whose hydrogen ion concentration was slightly above that of the blood were not convincing. If it is assumed that the alkalinity of the blood is lowered in septicemia the intravenous injection of an isotonic solution with a powerful buffer effect, like this dye solution proved to possess, would be expected to be most favorable for positive results. The best that could be obtained here was temporary check of the progress of a *Staphylococcus aureus* infection retarding death for less than two weeks. It is doubtful that injections with smaller doses of dye would have given much better results. There is much experimental evidence indicating that gentian violet in amounts of 7 to 11 mg per kilo, administered intravenously, fails to show any appreciable bactericidal action and can at best only be expected to give bacteriostatic effects. Considering the existing danger attending the intravenous use of dyes it seems that it ought to be possible to demonstrate decided bactericidal action in animal experiments before they are advocated for general intravenous medication. Dye is eliminated from the blood very rapidly and has at best only a short time for its maximum activity.



Controlling the osmotic pressure and hydrogen-ion concentration to the most desirable point known failed to reduce the toxicity of gentian-violet solution to any marked degree. This would perhaps also hold true for neutral acriflavine. The work of Burke and Newton<sup>4</sup> and Burke and Rodier<sup>5</sup> shows that the toxicity of gentian-violet and neutral acriflavine did not vary very markedly although their solutions varied in hydrogen-ion concentration from  $P_H$  5 to  $P_H$  8 and in osmotic pressure from less than one atmosphere to more than 12 atmospheres. There is still the possibility that the solvent may play an important part in toxicity. Hanzlik et al.<sup>6</sup> found that alkaline phosphates gave no serious reactions and were beneficial to the blood even when administered in slightly hypotonic and distinctly hypertonic solutions. The potassium phosphate solvents used here were isotonic and should cause no serious reactions intravenously. When mixed with one per cent gentian violet no perceptible chemical change seemed to take place. A fairly stable solution resulted, and it would be expected that if the dye were nontoxic the intravenous use of the dye solution would cause no greater reactions than the intravenous use of the solvent. The experimental evidence of this study indicated that the dye solution was toxic and in one case caused death as a result of the intravenous injection of a moderate dose. It seems that the toxicity was not due to the osmotic pressure nor to the hydrogen-ion concentration, nor even to the solvent, but to the gentian-violet itself. The toxic effect did not seem to be a function that was constant for the individual but seemed to vary in degree from time to time within the individual. This would be expected if symptoms and reactions rest fundamentally on disturbances in important physical and chemical mechanisms of the blood and tissue as suggested by Hanzlik et al.,<sup>6</sup> for minute but significant physical and chemical variations are bound to occur from time to time within the cells.

It seems that although osmotic pressure and hydrogen-ion concentration are important factors in intravenous dye therapy, toxicity and chemotherapeutic activity of gentian-violet, acriflavine, and dyes in general are strictly functions of the chemical constitution of the dye molecule and are controllable within limits by chemical manipulation. Therefore, future progress of intravenous dye therapy should have its greatest possibilities in the chemical manipulation of the dye molecule with a resulting increase in its chemotherapeutic activity and a decrease in its toxicity.

#### SUMMARY

1 Osmotic pressure and hydrogen-ion concentration determinations of several solvents and dye solutions used for intravenous injections showed wide differences in osmotic pressure and hydrogen-ion concentration.

2 Both the osmotic pressure and the hydrogen-ion concentration of gentian-violet and neutral acriflavine solutions may be controlled simultaneously to approximate those of the blood.

3 Gentian-violet was found to be a powerful buffer up to a point of about  $P_H$  7.6. Above this point the solution was unstable and broke down rapidly when potassium salts and sodium bicarbonate were used as solvents.

4 A one per cent isotonic, alkaline gentian violet solution did not seem to increase or decrease the toxicity of the dye to any appreciable extent when injected intravenously into the marginal ear vein of rabbits. The therapeutic value of the dye did not seem to be improved markedly by controlling the osmotic pressure and hydrogen ion concentration to a point closely approximating those of the blood.

5 The toxicity of gentian violet was found to be a function which is not constant within the individual but which may change in degree from time to time.

6 It is suggested that although osmotic pressure and hydrogen ion concentration are important factors in dye therapy the toxicity and chemotherapeutic activity of the dye are primarily functions of the chemical constitution of the dye molecule and can best be controlled by chemical manipulation of the molecule.

## REFERENCES

- <sup>1</sup>Brill J C and Meyers H B. Mercurochrome 220 Soluble and Gentian Violet, Jour Am Med Assn, 1925, lxxxiv 879
- <sup>2</sup>Browning, O H, Gulbransen R, and Kenneway E L. Hydrogen Ion Concentration and Antiseptic Potency With Special Reference to the Action of Acridine Compounds Jour Path and Bacteriol 1919 xxiii 106
- <sup>3</sup>Burke, Victor, and Grieve Irene. The Value of Alkali in Dye Therapy, Am Jour Med Sc, 1924 clviii, 98
- <sup>4</sup>Burke, Victor, Newton, Jane L and Sprague Aileen. Preparation of Gentian Violet for Intravenous Injection, Jour Am Med Assn 1926 lxxxvi 529
- <sup>5</sup>Burke Victor, and Rodier, E A. Preparation of Neutral Acriflavine Solution for Intravenous Injection. JOUR LAB AND CLIN MED 1927 xiii 231
- <sup>6</sup>Eggerth A H. The Bacterial Action of Acridine Dyes and the Adjuvant Effect of Serum, Jour Infect Dis, 1926 xxxviii, 440
- <sup>7</sup>Graham Smith, G S. Some Factors Influencing the Actions of Dyes and Allied Compounds on Bacteria, Jour Hyg 1919 xviii 1
- <sup>8</sup>Haazlik P J, De Ede F, and Tainter M J. Blood and Symptomatic Changes Following Intravenous Administration of a Variety of Agents and Solutions. Arch Int Med, 1925 xxx, 447
- <sup>9</sup>Harris, J A, and Gortner R A. Notes on the Calculation of Osmotic Pressure of Expressed Vegetable Saps from the Depression of the Freezing Point With a Table for the Values of P for  $\Delta$  0.001 to  $\Delta$  2.999. Am Jour Bot 1914 i 75
- <sup>10</sup>Klugler I J. A Study of the Antiseptic Properties of Certain Organic Compounds, Jour Exper Med, 1918, xxvii 46.
- <sup>11</sup>Meleney, F B and Zau, Zung Dau. Action of Acriflavine on the Blood and Certain Tissues of Rabbits, Jour Am Med Assn 1925 lxxxiv 337
- <sup>12</sup>Reed, A C and Lum D D. Toxic Results of Dye Treatment in Septicemia, Jour Am Med Assn, 1924, lxxxiii 1681
- <sup>13</sup>Spencer Harvey. Effects of Intravenous Injections of Acriflavine. JOUR LAB AND CLIN MED 1924, ix 322
- <sup>14</sup>Young, H H and Hill Justinia H. Treatment of Septicemia and Local Injection by Mercurochrome 220 Soluble and by Gentian Violet Jour Am Med Assn, 1924 lxxxii, 669

# INCREASING WEIGHT WITH INSULIN\*

## PRELIMINARY REPORT

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RELATIVELY little is to be found in the literature of this country regarding the use of insulin in the treatment of individuals who for one cause or another are underweight. In 1924 Mairrott<sup>1</sup> and Barbour<sup>2</sup> each published an article on the use of insulin in malnutrition of childhood. An article on the same subject was published by Tisdall et al.,<sup>3</sup> in 1925 and one by Fischer and Rogatz<sup>4</sup> in 1926. These all concerned the use of insulin in malnutrition of childhood. Careful search of the literature has not revealed any reports of its trial in adult malnutrition in this country, although many such references are to be found in the foreign literature and especially that of Germany and France. Such reports can be briefly summarized by stating that the results were almost universally successful in the attempt to force feeding and increase weight in nondiabetic malnutrition. It has been found especially valuable for building weight and aiding convalescence in pulmonary tuberculosis. Although it would be impossible in the brief scope of this article to relate the various experiences reported by foreign observers,<sup>5</sup> one or more references may be cited.

Vogt<sup>6</sup> believes with Falta<sup>7</sup> that insulin affects not only carbohydrate metabolism but also the general metabolism, and accelerates intestinal digestion and absorption, he has found it of value in forced alimentation. Schellong and Hufschmid<sup>8</sup> summarize the progress in this direction and give the experience of their own clinic. They believe the class of patient to benefit by this treatment is affected with constitutional leanness and small appetite. Their experience would indicate that a condition of equilibrium is reached toward the close of the second week, as apparently the subject becomes in part habituated or immunized against the further action of insulin. Moirni and Bouessée<sup>9</sup> obtained rapid weight increase in patients with pulmonary tuberculosis. They believe it of real value in this condition.

From these references and my experience it would appear that insulin can occupy a place of first importance in the therapeutics applied against malnutrition generally. A brief tabulation and description of several cases<sup>10</sup> so treated follow. Although the series is not extensive, results have been quite definite in all, and enough experience has been gained to make it possible to offer a few suggestions in the use of insulin for weight-producing purposes.

### REPORT OF CASES

CASE 1—G. F., female, aged twenty one, admitted to medical service July 6, 1927. History of progressive loss of weight over long period with anorexia, but no definite localizing

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of pathology The patient had been in Mt Sinai Hospital from November 29, 1926, to January 16, 1927, discharged improved, diagnosis inanition

Signs suggesting consolidation of right apex, but no râles X ray showed slight general hypervascularization but otherwise negative Verne's test for active tuberculosis negative Sputum negative Temperature normal or subnormal throughout, except from July 13 to August 3, when there was a slight P.M. rise Erythrocytes 4,410,000, hemoglobin 14.5 gm (88 per cent) Leucocytes 4,500, polynuclears 57 per cent, lymphocytes 40 per cent Glucose tolerance test normal Blood chemistry urea nitrogen 9.8, sugar 72, cholesterol 137, chlorides 478 Diagnosis malnutrition, chronic pulmonary tuberculosis, anxiety neurosis

## CASE 1

DATE 1927	WEIGHT	DIET	D P	BASAL METAB	REMARKS
July 6	60	Regular		-42	Extreme weakness and emaciation
8		"			
9		"			
12		"		-43	
15	55½	"			
16	55½	High caloric with intermediate feedings			
22	56½	"			Thyroid extract gr 3 daily begun
25	58	"			Ovarian extract gr 5 added
27	57½	"			
29	58½	"	75/55		
Aug 1	57	"			
2	55	"			
3	55	"			
6	58	"			
12	61	"	80/56	+7	
19		"			
22		"			Insulin U 3 t i d 15 mm a c begun
26	66	"			
29		"			Insulin increased to U 5 t i d a c
Sept 2	73	"			General condition much better Ont of bed and much more cheerful
9	79	"			
13		"	98/60		
16	85	"			Discharged from hospital in very good physical condition Later observation showed further improvement Insulin discontinued on leaving hospital
23	90	"			

CASE 2—M S, female, aged seventy four Pneumonia twenty one months previously Under treatment for mastoid disense since Very poorly nourished Initial weight 100 pounds Permanent improvement in health after insulin Ambulatory

## CASE 2

DATE 1927	WEIGHT	DIET	REMARKS
Oct 25	100	Mixed	Began taking insulin U 5 before breakfast and supper only
Nov 2	102	Mixed	Dose increased to U 8 b i d Appetite increased, more food consumed
4			
11	104	Mixed	Insulin U 8 b i d continued
15	106	Mixed	Third week of insulin administration completed Feeling much better and stronger

CASE 3—B McC male, aged twenty six. Under observation for two years for general poor health, underweight, furuncles, Vincent's angina, etc. Small discharging sinus in gluteal region No signs of pulmonary tuberculosis at any time Weight in October, 1925 was 115½ pounds Ambulatory

## CASE 3

DATE 1927	WEIGHT	DIET	REMARKS
Oct 17	115¾	Mixed	Insulin U 6 tid ½ hr ac prescribed
25	120¼	Mixed	Reported inordinate hunger following insulin, and in gestion of unusual amounts of food
Nov 8	117¾	Mixed	No insulin previous three days and only irregular doses before that Becoming irritated by repeated injections 8 units bid before breakfast and supper ordered
17	119¾	Mixed	8 units bid ½ hr before breakfast and supper P 76, B P 96/66 Insulin discontinued No permanent improvement in weight though general health better

CASE 4—E M, male, aged sixty one Secondary anemia and malnutrition following acute bronchitis Approximately 55 pounds under average weight for age and height Ambulatory

## CASE 4

DATE 1927	WEIGHT	DIET	ERYTHROCYTES	HEMO *	REMARKS
Oct 14	118½	Mixed regular	3,200,000	65	Rather feeble Lungs clear In suln U 5 tid ac prescribed
20	122	Mixed regular			Dose increased to U 8 tid ac
28	123	Mixed regular			To continue U 8 tid ac
Nov 4	124½	Mixed regular	3,980,000	72	To continue U 8 tid ac
11	125	Mixed regular	3,960,000	72	To continue U 8 tid ac
17	127	Mixed regular	4,100,000	75	To continue U 8 tid ac
25	127½	Mixed regular			To continue U 8 tid ac
Dec 2	127½	Mixed regular	4,380,000	78	Insulin discontinued this date Only moderate stimulation of appetite by insulin during period
9	127	Mixed regular			No insulin past week

CASE 5—M K, female, aged seventy Chronic "indigestion" Visceroptosis Ap proximately 50 pounds under average weight for age and height Ambulatory

## CASE 5

DATE 1927	WEIGHT	DIET	REMARKS
Oct 25	110	Mixed	Insulin U 5 b.i.d before breakfast and supper pre scribed
26	110	Mixed	
Nov 2	108¾	Mixed	Increased appetite noted
4		Mixed	Dose increased to 8 units b.i.d
11	107½	Mixed	Eating more heartily
15	109¾	Mixed	Three weeks of insulin completed Patient says that she consumed much candy during period Possible cause of failure

CASE 6—G C, female, aged forty one, admitted to medical service September 23, 1927, discharged, improved, November 6, 1927 Diagnosis visceroptosis, chronic pulmonary tuber culosis

There was a regular afternoon rise in temperature of one half degree during stay in hospital X ray showed old central tuberculous nodules with root branch thickening and general hypervascularization Congestion was especially noted in right upper lobe There was no definite x ray evidence of recent activity Verne's test for active tuberculosis was negative Urine was essentially negative on repeated analysis Fasting blood sugar on November 3 was 80 mg Basal metabolism on September 29, 3 per cent below average normal On September 24 erythrocytes numbered 4,296,000, leucocytes 8,500 Gastric analysis on September 26 was normal

## CASE 6

DATE 1927	WEIGHT	DIET	REMARKS
Sept 23	86	Regular	Admission
27		Regular	Insulin begun U 5 at 8 30 A M and 4 30 P M
28		High calorie	
30	90	High calorie	
Oct 4	90	High calorie	
6	90	High calorie	
11		High calorie	Insulin increased to U 5 at 8 and 11 A M and 4 P M
13	91	High calorie	Insulin increased to U 5 at 9 A M U 8 at 11 A M and 4 P M
14	96	High calorie	
15 20	96	High calorie	Blood pressure on October 18 110/65
21	97 1/2	High calorie	
22	96 1/2	High calorie	
24	96 1/2	High calorie	
25	97 1/2	High calorie	Insulin increased to U 8 at 8 A M, 11 A M and 4 P M
26	98 1/2	High calorie	
27	100	High calorie	
28	101	High calorie	Blood pressure 130/80
29	100	High calorie	
31	100	High calorie	
Nov 1	101	High calorie	
2	101	High calorie	
4	102	High calorie	
5	101 1/2	High calorie	Insulin discontinued patient discharged Nov 6

CASE 7—H H, female aged thirty three Average weight during adult years has been between 90 and 95 pounds and often as low as 88 pounds She has been in fair health in the past except for a severe attack of septicemia lasting from November, 1925 to May, 1926 Recovery was complete and health has been as before Easily fatigued Appetite poor most of time Eats very little breakfast if any, and only small portions at other meals Weight in December, 1927 91 pounds

This patient was started on 10 units of insulin b.i.d. half hour before breakfast and before supper She was followed for only a few days as she was obliged to leave the city, and is mentioned in this article only because of the astonishing effect produced upon her appetite and attitude toward food A typical breakfast after insulin was a large portion of oatmeal with cream, a half grapefruit, one fried egg two thick slices of bread with butter, and a piece of cake An enormous dinner was likewise consumed following the second dose at evening It seems inevitable that this subject would have gained considerable weight if this routine had been continued

## COMMENT

The foregoing case reports indicate that insulin is without doubt of value in increasing weight in conditions other than diabetes, or in other words where it can be assumed that the pancreas is normal and there is no diminution of sugar tolerance The results obtained in Case 1 are perhaps the most striking as here the percentage gain was greatest and the control period the longest Later experience has suggested that greater success might have been obtained in Cases 2, 4, and 5 if more attention had been given to proper food selection

Whether the total glycogen of the body is increased or decreased by the administration of insulin to normals (the literature contains rather contradictory evidence on this point the weight of evidence favoring a decrease) it nevertheless is true that an individual can through insulin be induced to eat and assimilate a large amount of food, much of which will later be deposited as fat This is the fact of practical importance in the use of insulin

sulin for weight production. It is essential to take fullest advantage of the hunger engendered by the insulin. For this purpose a food mixture in which there is a large proportion of fat would seem logical. As a relatively small amount of sugar is sufficient to antidote promptly a dose of insulin and thus inhibit the food craving, it would seem that, in the attempt to increase weight, a food combination low in sugar but of a high caloric value in relation to weight and bulk should be selected. It is probable that the failure to increase weight in Case 5 was the result of eating much candy during the insulin period.

The dietary suggested above is almost the direct antithesis of that advocated by Schellong and Hufschmid, who advocate the giving of fairly large amounts of bread and honey or sugar both before and after the insulin injection. It would seem that this procedure is failing to capitalize on the intense food craving to the greatest extent by inducing the subject to partake of the greatest possible amount of fat-producing foods. In the majority of underweight cases it is probably not the alteration of metabolism which is desired primarily, but rather the ingestion of a larger amount of food. Insulin accomplishes this purpose. It is very questionable if Case 7 would have consumed as great quantities of food following insulin if sugar had been permitted before and after.

Psychic aversion to food as frequently encountered in the psychasthenic can be most successfully treated by insulin. In Cases 1 and 7 there was produced a change from anorexia with almost complete indifference to food to a marked relish and avidity. Experience has indicated that the optimal time for eating after a dose of insulin is about thirty minutes, as this interval usually produces the hunger and intense food craving without other alarming symptoms associated with hypoglycemia. A patient should always be warned, however, of what these symptoms are and how to combat them, as in the treatment of diabetes. Although the dose in this series was never increased above ten units t.i.d. I do not see any objection to doing so provided there is little or no response to smaller doses and the patient is sufficiently guarded against hypoglycemic shock. A better result could possibly have been obtained in Case 4 had a larger dose been administered.

Little can be said at present of maintaining weight once it has been acquired. This, however, would seem to be a relatively simple and easy matter. A better habit of eating having been established during the insulin period, it can be expected that the individual will continue to ingest sufficient food to maintain the increase. If necessary, an occasional short course of insulin therapy may be given.

The effect of insulin on the normal metabolism has not been studied as thoroughly as on that of diabetes. In diabetes the glycogen store of the liver is known to be increased by it, as is also that of depancreatized dogs, when sugar is fed. Bailey<sup>11</sup> found no increase in the basal rate after insulin, although an increase in the respiratory quotient is commonly observed, indicating an increased oxidation of carbohydrate. MacLeod<sup>12</sup> states that there is an "undoubted decrease in total glycogen which occurs on injecting insulin into

normal animals" and that there must remain a fraction of available carbohydrate which changes into other metabolites, as not all is accounted for by oxidation and glycogen formation. However this may be fat is produced in malnutrition under the influence of insulin. This is due in part, no doubt, to increased food intake, perhaps also in part to a direct influence of the insulin on general metabolism.

## SUMMARY

1 Of seven cases of malnutrition treated with insulin all showed increased appetite and some had intense food craving following insulin, five showed definite gains in weight in response to insulin one showed no gain whatever but was slightly under the original weight at the end of three weeks, one was not observed a sufficient length of time.

2 It is concluded that insulin can be a valuable agent for increasing weight in malnutrition.

3 Attempts should be made to increase the fatty as well as the starchy foods after the administration of insulin when malnutrition is treated.

4 Thirty minutes should elapse after insulin administration before food is taken if the optimum development of appetite is desired.

5 The insulin should be given three times a day before meals in doses of 10 units more or less according to individual indications.

Since this article was prepared an article<sup>13</sup> bearing on the same subject has appeared in the literature. The authors conclude that "insulin treatment properly controlled is a valuable adjunct in the treatment of certain critical cases of undernutrition in psychotic patients." They obtained usually a marked increase of weight with insulin.

## REFERENCES

- <sup>1</sup>Marriott, W. McKim. The Food Requirements of Malnourished Infants. With a Note on the Use of Insulin. *Jour. Am. Med. Assn.* 1924 lxxxix 500.
- <sup>2</sup>Barbour, O. Use of Insulin in Undernourished Non-diabetic Children. *Arch. Ped.*, October, 1924 xli, 707-711.
- <sup>3</sup>Tisdall, F. F., Brown, A., Drake, T. G. H., and Cody, M. G. Insulin in Treatment of Malnourished Infants. *Am. Jour. Dis. Child.*, July, 1925, xxx 10-18.
- <sup>4</sup>Fischer, L., and Rogatz, J. L. Insulin in Malnutrition. *Am. Jour. Dis. Child.*, March 1926, xxxi, 363-372.
- <sup>5</sup>A complete bibliography is too extensive for inclusion here. Complete references to the foreign literature are readily available through the Quarterly Cumulative Index of the past few years under the heading "Uses of Insulin."
- <sup>6</sup>Vogt, E. Dietotherapy. Especially on Forced Alimentation with Insulin Pills. *Med. Klin.* June 18, 1926 xxi, 950-952.
- <sup>7</sup>Falta, W. Lennenes and Insulin Patterning. *Wien. klin. Wchnschr.*, (supp. no. 13) 1926, xxxix 1-15.
- <sup>8</sup>Schellong and Hufschmid. Über die Wirkungsweise der Insulininjektionen bei Mastkuren und über eine dabei zu beobachtende scheinbare Gewöhnung an Insulin. *Klin. Wchnschr.* October 1, 1927 xl 1888.
- <sup>9</sup>Morini, J., and Bouessée, F. La Cure d'engraissement Par L'insuline Dans La Tuberculose Pulmonaire. *Annales de Médecine.* October 1927 xxi 308.
- <sup>10</sup>Dr. Edward King first suggested the use of insulin in the case of G. F. of this series which formed the basis of the present study.
- <sup>11</sup>Bailey, Cameron V. Private communication.
- <sup>12</sup>McCleod, J. J. R. *Physiology and Biochemistry in Modern Medicine*, ed. 5, The C. V. Mosby Company, St. Louis, 1926.
- <sup>13</sup>Appel, Kenneth E. et al. Insulin Therapy in Undernourished Psychotic Patients. Preliminary Report, *Jour. A. M. A.*, 1928 xc No. 22, p. 1788.



# THE ADAPTATION OF STANDARD ANTIGENS TO THE PRECIPITIN TEST FOR SYPHILIS, WITH COMPARATIVE RESULTS SHOWN BY THREE THOUSAND SERA\*

By H W BUTLER, M.D., NEW ORLEANS

THE principles governing the reactions of the different precipitation tests are essentially the same, so that when a particular method of performing the test is desired, the antigen is modified in such a way as to be applicable to that particular technic

In a recent article,<sup>1</sup> I reported on the comparative results obtained with the Kahn precipitation test for syphilis, showing an agreement of 95.8 per cent with the standard Wassermann reaction. By the use of the Meinicke technic on a series of 500 cases, the percentage of agreement was found to be 97.5 per cent. In addition to the close agreement with the Wassermann and to the simplicity of the performance of the test, the sharp demarcation between the negative and positive reactions makes the test valuable by this method. The use of an acidulated or alkalinized antigen containing a gum resin flocculation indicator, as proposed by Meinicke, possesses distinct advantages over other methods of performing the precipitation test. As horse hearts used by Meinicke are difficult to obtain in America, I wish to present methods of adapting our standard beef heart antigens for use with this test and to show a comparative series of the results obtained with such modifications.

## RESULTS OBTAINED BY USING MEINICKE TECHNIC AND THE HORSE HEART ANTIGEN PREPARED BY MEINICKE

One c.c. of the horse heart antigen is measured into a twenty c.c. test tube. Ten c.c. of a 3 per cent saline solution is measured into a second similar tube. Both tubes are placed in the water-bath at 45° C. for five to ten minutes, after which the contents of the tubes are poured together twice. One c.c. of this suspension, while warm, is pipetted into a test tube into which has been previously placed 0.2 c.c. of blood serum, not inactivated. The tubes are shaken and allowed to stand at room temperature overnight. Reading. The strong positive sera appear crystal clear with the sediment at the bottom of the tube. The moderately positive sera appear hazy with a considerable amount of sediment at the bottom. The weakly positive sera are slightly cleared with a small amount of sediment at the bottom of the tube. The negatives appear as when first set up, "milky," with no sediment.

### SPINAL FLUIDS, HORSE HEART ANTIGEN (MEINICKE)

One-half c.c. of the emulsion, prepared as for blood sera, is pipetted into a tube containing 0.5 c.c. of spinal fluid and allowed to stand overnight.

\*From the Department of Medicine, Tulane University School of Medicine.  
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Strongly positive spinal fluids will settle out in the same way as the blood sera, but the microscope is necessary in most cases. A characteristic clumped precipitate appears in the positives and the negatives show no such precipitate.

COMPARATIVE RESULTS ON BLOOD SERA USING THE KOLMER WASSERMANN ANTIGEN  
AND THE ORIGINAL MEINICKE ANTIGEN

<i>Wassermann</i>	<i>Meincke</i>
72 Strongly positive	Strongly positive
5 Negative	Strongly positive
3 Negative	Moderately positive
8 Moderately positive	Strongly positive
6 Strongly positive	Moderately positive
1 Weakly positive	Strongly positive
4 Weakly positive	Moderately positive
1 Moderately positive	Moderately positive
2 Moderately positive	Negative
1 Weakly positive	Weakly positive
1 Anticomplementary	Weakly positive
2 Anticomplementary	Moderately positive
436 Negative	Negative
<u>542</u>	

COMPARATIVE RESULTS ON SPINAL FLUIDS USING KOLMER WASSERMANN AND ORIGINAL MEINICKE

<i>Wassermann</i>	<i>Meincke</i>
11 Strongly positive	Strongly positive
3 Weakly positive	Weakly positive
2 Weakly positive	Moderately positive
2 Negative	Moderately positive
	(Negative globulin)
	(Cell count—1)
1 Weakly positive	Strongly positive
65 Negative	Negative
<u>84</u>	

KOLMER ANTIGEN

Kolmer's Wassermann antigen can be changed into a precipitation antigen by diluting and adding a 5 per cent tincture of tolu (made from the balsam) as an indicator.

Antigen	5 c c Kolmer antigen
	25 c c Alcohol, 95 per cent
	10 c c Tincture of tolu, 5 per cent
Diluting saline	100 c c distilled water
	3 gm sodium chloride
	0.15 c c of 10 per cent sodium hydroxide

Some antigens require more alkali than others, depending upon the concentration of lipoidal substance. Enough antigen should be added to this formula to reach the point of saturation for that particular antigen, the object being to make the suspension as unstable as possible without producing a

precipitate in the negative sera. It should be added to the point just short of this amount. The more antigen that can be added, the more sensitive the reaction, and the more alkali that is added, the less sensitive it is. The correct amount of antigen is obtained by testing against negative sera. The amount ranges from 2 to 5 c c if the Kolmer<sup>2</sup> method of extraction is used. The alkali is necessary to render the ether extract portion of the antigen suitable for emulsification.

One c c of the antigen is measured into a 20 c c test tube, and 10 c c of the alkaline saline measured into a second 20 c c test tube. These are placed in the water-bath at 56° C for five minutes. They are then poured together twice. One c c of this suspension is measured immediately, while still warm, into a test tube containing 0.2 c c of blood serum which has not been inactivated. The suspension precipitates when it becomes cold, unless serum is added.

*Spinal Fluids*—A Purdy tube is filled to the three c c mark with spinal fluid. Two c c of a saturated solution of ammonium sulphate are added and well mixed. It is allowed to stand at room temperature for fifteen minutes and then centrifuged.<sup>3</sup> The supernatant fluid is pipetted off, and the globulin is taken up in 0.2 c c of negative serum. One-half c c of the suspension as used for blood sera is added.

#### RESULTS OBTAINED WITH THE KOLMER WASSERMANN ANTIGEN, USING IT IN BOTH TESTS

<i>Wassermann</i>	<i>Precipitation</i>
43 Strongly positive	Strongly positive
11 Moderately positive	Moderately positive
3 Weakly positive	Moderately positive
2 Weakly positive	Negative
1 Negative	Moderately positive
6 Moderately positive	Strongly positive
1 Weakly positive	Moderately positive
6 Weakly positive	Weakly positive
2 Anticomplementary	Negative
2 Strongly positive	Negative
4 Anticomplementary	Strongly positive
489 Negative	Negative
<u>570</u>	

*Beef Heart Extraction*—Fresh veal hearts, the size of a small fist, are selected and the auricles and superficial fat removed. The muscle is ground in a sausage grinder and spread on paper and dried by an electric fan. After it is completely dried, it is reground and extracted with ether as follows. Four hundred c c of ether are used to each 100 gm of dry ground heart and allowed to act for ten minutes, shaking frequently. The ether is filtered off and 300 c c are added three different times to the heart muscle and treated in the same manner. The heart muscle is now dried completely free from ether, and for each gram of muscle, 5 c c of 95 per cent alcohol are added and

maceration is allowed to continue for three days at room temperature after which the alcohol is filtered off and made up to the original volume with 95 per cent alcohol

Some of this extract is saturated with cholesterol (to 100 c c add 0.6 gm of cholesterol)

*Veal Heart Formula —*

Veal heart extract	15 c c
Veal heart extract, cholesterolized	15 c c
Alcohol 95 per cent	15 c c
Tincture of tolu 5 per cent	15 to 20 c c
Alcohol containing 1 per cent benzoic acid	1 c c

Tolu varies. Add enough to the antigen to produce sufficient opacity when diluted. Too much tolu will obscure the precipitation. One c c should require 0.5 c c of N/10 NaOH for neutralization. Phenolphthalein is used as an indicator. The  $P_{11}$  is 4.8.

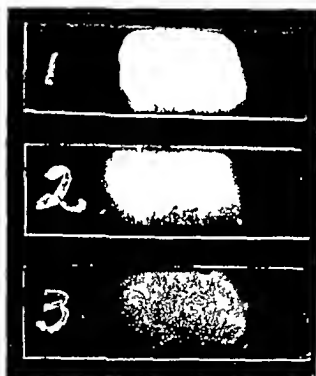


Fig 1—Butler slide method. No 1 negative. No 2 moderately positive. No 3 strongly positive.

*Formula —*

Veal heart extract	200 c c
Alcohol 95 per cent (containing 0.6 gm cholesterol)	100 c c
Alcohol 95 per cent (containing 5 per cent of the balsam of tolu)	110 c c
Alcohol 95 per cent (containing 1 per cent benzoic acid)	5 c c
Mix and filter. This formula needs no adjusting.	

*Diluent —* 3 per cent saline

One c c of antigen is measured into a 20 c c test tube and 10 c c of 3 per cent saline into a second 20 c c test tube, heating in a water bath as before at 56° C two to five minutes. Heating at 56° C for more than five minutes decreases

rapidly the sensitiveness of the test. Mix by pouring back and forth several times. One c c is measured into a test tube containing 0.2 c c of blood serum (not inactivated). Reading is made after standing overnight at room temperature.

*Slide Test*—If the slide method is desired, the antigen is diluted only three times, i. e., 0.1 c c antigen and 0.3 c c of 3 per cent saline. A one c c pipette, as short as can be obtained, is fitted with a Wright's rubber bulb. The antigen is measured into a test tube with the dry pipette, and then, with the same pipette, 0.3 of 3 per cent saline is taken up and forced into the test tube containing the antigen. The mixture is sucked back and forth several times. No heating is required. Three drops of clear serum, using a capillary pipette, are placed upon a slide and three drops of the antigen mixture are placed alongside of the serum. It is mixed with a toothpick, and the slide rocked several times to insure thorough mixing. It is then laid aside for five or ten minutes, or longer, depending upon the amount of drying that takes place. As the preparation dries, it passes through different degrees of concentration, so that at some stage the zone of inhibition will be missed and precipitation allowed to take place. The positive bloods will show the characteristic precipitation, but the negatives will remain granular. The slide method should be performed at room temperature. It is slightly more sensitive than the tube method, and the reading is more striking.

*Spinal Fluids*—The globulin is concentrated, using the Kahn technic<sup>3</sup> as for the Kolmer antigen. It is dissolved in 0.2 c c of normal saline or negative serum and one-half of a c c of suspension 1 to 10 is added. Strong positive spinal fluids can be read on a slide in the same way as a blood serum. Concentration is necessary in most cases, using 3 drops of concentrated globulin and 3 drops of the 1 to 3 suspension.

#### RESULTS WITH THE VIAL HEART ANTIGEN AND THE KOLMER WASSERMANN SYSTEM

<i>Wassermann</i>	<i>Precipitation</i>
186 Strongly positive	Strongly positive
1 Strongly positive	Negative
1 Weakly positive	Negative
1 Negative	Strongly positive
4 Anticomplementary	Strongly positive
1 Negative	Moderately positive
9 Moderately positive	Strongly positive
10 Weakly positive	Strongly positive
10 Negative	Weakly positive
3 Weakly positive	Weakly positive
3 Strongly positive	Moderately positive
5 Moderately positive	Moderately positive
2 Strongly positive	Weakly positive
1776 Negative	Negative
<u>2012</u>	

*Comments*—The Mehncke reaction, in its present form, is the nearest approach to the Wassermann in specificity of any of the precipitation tests in my experience. It is easy to perform. 0.2 c c of raw serum is measured into

a test tube, and 1 c.c. of the warm antigen suspension added. The tube is shaken and allowed to remain at room temperature overnight. It offers no difficulty in reading. If there is any criticism to make, it is that the horse heart extraction may be a little too sensitive. There is frequently a quantitative disagreement between the Wassermann and the precipitation reactions. A weakly positive Wassermann may give a strongly positive precipitation and a strongly positive Wassermann may give a weakly positive precipitation. Occasionally, one reaction will be strongly positive and the other negative. Two Wassermann systems were used in checking the precipitin tests in many of these cases.

*Conclusions*—The Meinicke precipitation technic with some variations, can be applied to any of the standard antigens and the results obtained are commensurate with the sensitiveness of the antigen used. It is easy to perform and requires the minimum amount of time and laboratory facilities. Inactivation of the serum is unnecessary. There is no difficulty encountered in the interpretation of the results which show a high degree of accuracy as compared with the Wassermann reaction.

NOTE—This work was made possible by the excellent cooperation of Dr. G. H. Hauser and the entire staff of the Pathological Department of Charity Hospital, New Orleans, whose Wassermann system was checked.

#### REFERENCES

- <sup>1</sup>Butler, H. W. A Simple Rapid Precipitin Test for the Diagnosis of Syphilis. New Orleans Med. and Surg. Jour. August 1926 lxxix 105.
- <sup>2</sup>Kolmer, John A. and Boerner, Fred. Laboratory Diagnostic Methods. New York: D. Appleton & Co. 1925.
- <sup>3</sup>Kahn, R. L. Serum Diagnosis of Syphilis by Precipitation. Baltimore: Williams & Wilkins Company. 1925.

# THE CONSTITUTION OF NEOARSPHENAMINE\*

## A NEW STRUCTURAL FORMULA

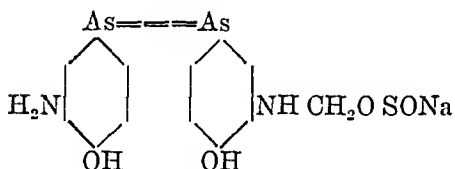
BY BERNARD SALKIN, NEW YORK CITY

DURING an investigation on neoarsphenamine, a number of samples were examined by the method outlined by Macallum,<sup>†</sup> and it was found that if the calculations were made according to the method outlined in that article, the results were wrong on their face

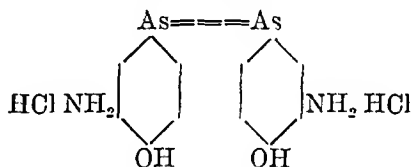
It was found that the arsenic in neoarsphenamine requires only *two* iodines for oxidation while the arsenic in arsphenamine actually requires *four* iodines for its oxidation. This led to a further investigation of the constitution of the compound, and the following results were arrived at

1 There seems to be a regulation between the "sulphoxalate content" of the neoarsphenamine and the toxicity of the product,

2 The constitutional formula for neoarsphenamine, namely,



which was probably arranged



by analogy to arsphenamine,

and which is consistently found in the literature, does not express the facts,

3 The arsenic in neoarsphenamine is linked in a way that its oxidation from the tri- to the pentavalent state is "divalent," while the oxidation of the arsenic in arsphenamine (from the tri- to the pentavalent state) is "tetra-valent"

The method used for the examination of the samples was that outlined by Macallum (cited above) and is essentially as follows

Two grams of neoarsphenamine are dissolved in 100 ml of water

a Ten ml of this solution was acidified, and N/10 iodine solution added immediately. The excess was titrated back after three minutes with N/10 sodium thiosulphate solution

b Twenty ml of the same solution was acidified. The free "sulphinic acid" (neoarsphenamine "acid") was filtered off, and to an aliquot portion of the filtrate, N/10 iodine solution was added. After three minutes the excess was titrated back with N/10 thiosulphate solution

\*From the Research Laboratories Travis Colloid Research Company N Y

†Jour Am Chem Soc 1920 XLIII 643

c The arsenic content was determined in 10 ml of the solution

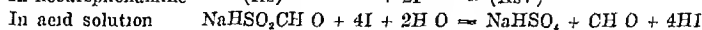
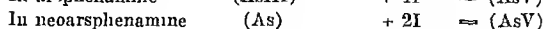
#### Notes

1 The results are all reduced to a basis of ml of N/10 iodine per gram of neoarsphenamine

2 Calculations were made by the slide rule

The atomic weight for arsenic was taken as 75, the molecular weight of neoarsphenamine as 466, and the molecular weight of sodium formaldehyde sulphoxalate as 118

The calculations were based on the following equations



3 All flasks were filled with nitrogen gas and the solution in (b) was filtered in a nitrogen atmosphere

4 The oxidation of arsenic to the pentavalent state is not complete in three minutes and this is reflected in the "combined sulphoxalate" result, giving a slightly higher result for the latter

The result obtained from (a) is the number of ml of N/10 iodine required for one gram of neoarsphenamine (total reducing power)

The result obtained from (b) is the number of ml of N/10 iodine required for the "Free Reducing Substances" in one gram of neoarsphenamine

From (c) we obtained the number of ml of N/10 iodine required to oxidize the arsenic in one gram of sample

Adding (b) plus (c) and subtracting the sum from (a), we obtain the number of ml of N/10 iodine required to oxidize the sulphoxalate in the precipitated "Neo Acid" in other words for the "combined sulphoxalate," (d)

The results are given in Table I

If we use the calculations based on the "tetravalent" oxidation of the arsenic, we obtain the following results

SAMPLE NO	THEORETICAL COMBINED SULPHOXALATE ON THE BASIS OF ARSENIC CONTENT	COMBINED SULPHOXALATE (AS CALCULATED)
	IN PER CENT	IN PER CENT
46	15.98	0.93
48	16.70	2.70
49	16.98	3.48
51	17.32	7.1
52	15.93	4.84
53	16.41	5.14
54	14.41	7.91
55	16.40	3.88
A	15.29	4.65
B	13.09	2.89

We must, therefore conclude that there is a difference in the way the arsenic in neoarsphenamine oxidizes from the oxidation of the arsenic in arsphenamine. Since in both cases the arsenic is oxidized to the pentavalent



TABLE I

NEO NO	C C N/10 A	IODINE SOL B	AS (75) PER CENT	C C N/10 C	IODINE SOL D	COMBINED SULPHO- ALATE (118) PER CENT	THEORETICAL	EXCESS COMBINED SULPHO- ALATE OVER THEORY PER CENT
							COMBINED SULPHOX ALATE ON BASIS OF ARSENIC CONTENT PER CENT	
46	149.2	37.55	20.36	54.20	57.45	16.92	15.98	0.94
48	157.0	34.65	21.22	56.70	65.65	19.38	16.70	2.68
49	173.0	46.10	21.60	57.55	69.35	20.41	16.98	3.43
51	183.0	53.50	22.02	58.80	70.70	20.82	17.32	3.50
*52	174.5	50.00	20.30	54.10	70.40	20.75	15.93	4.82
53	178.6	49.70	20.92	55.75	73.15	21.60	16.41	5.19
*54	172.9	50.45	18.38	49.00	73.45	21.61	14.41	7.20
55	179.0	54.45	20.90	55.75	68.80	20.28	16.40	3.88
*A	159.0	39.30	19.45	51.90	67.80	20.00	15.29	4.71
B	132.0	33.40	16.65	44.45	54.15	15.99	13.09	2.90
†	158.2		30.30	161.50†				

\*Very toxic

\*A "Neo" precipitated by methyl alcohol

B "Neo" made by a different process

†The oxidation of As is not complete within three minutes

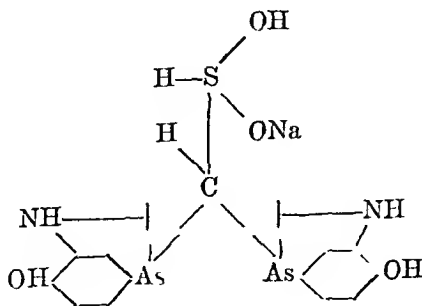
‡Arsphenamine

state, we must conclude that, whereas, in arsphenamine the two arsenic atoms are linked by a double bond, in the case of neoarsphenamine, the arsenic atoms are either bridged by a group which allows a normal oxidation to take place or they are not linked at all.

We thus come to the consideration of a new constitutional formula for neoarsphenamine.

While we have experimental proof that the formula is *not* as at present accepted, the new formula suggested is more in the nature of a hypothesis, and for the stimulation of further research.

Because of the results obtained by oxidation, we may assume that the following is the case:



Closing of the amine groups may be assumed from the fact that on precipitating neoarsphenamine with hydrochloric acid, no hydrochloride of the amine is formed. *The precipitate contains no chlorides.* This is directly opposed to the case of arsphenamine where two HCl groups are taken up before a neutral product is obtained. Another interesting observation made on the "sulphuric acid" is, that whatever be the composition of the original neo-

arsphenamine (as long as it is a monosulphoxalate) the precipitated "neo acid" will contain the theoretic proportions of carbon, hydrogen, sulphur, and arsenic

Another explanation for neoarsphenamine might be that the sulphoxalate becomes an addition product of the arsenic (Cf silver arsphenamine)

A third possibility is that where the sulphoxalate is attached to one arsenic atom only, and the internal ring is closed through the NH groups

It is hard to believe that the present formula has been accepted in view of the three outstanding facts which oppose such a formula

1 The fact that even a large excess of HCl does not precipitate a "hydrochloride"

2 The "divalent" oxidation is opposed to a double linking of the two arsenic atoms

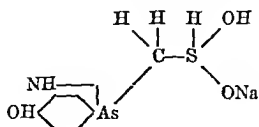
3 It is hardly conceivable that whereas neoarsphenamine, the "sulphimic acid" of which contains the theoretic amount of sulphoxalate to form the "monosulphoxalate" is nontoxic the additional content of sulphoxalate, which is itself not toxic, causes toxicity of a high degree. As the sulphoxalate is not toxic its further addition to arsphenamine in a position similar to that in which the first group had been added the amine group, should not form a toxic product

This brings us to a consideration of toxicity

The sample No 46, was the only one of the group examined which was not toxic. In this sample the relation of sulphoxalate to arsenic is nearly theoretical (It is probably closer to theory than the result shows)

In the case of the other samples and they were all toxic there is more sulphoxalate than the theory calls for in a monosubstitution product. This means that there is actually present five times as much disubstitution product as the percentage of excess sulphoxalate. For example, sample No 53, analyzing 5.19 per cent excess sulphoxalate, probably contains 25 per cent of the disubstitution product, and thus would be very toxic

If we assume the new formula for neoarsphenamine, we can understand the reason for a toxic compound being formed in the case of the disubstitution product. As soon as the second sulphoxalate group gets into the molecule, the arsenic atoms are no longer linked with one another, but break apart, each one probably having a sulphoxalate group attached to it or what is even more probable, the arsphenamine molecule is broken into two similar molecules, each having only one benzene ring, e.g.



## SUMMARY

1 The relation of combined sulphoxalate to arsenic (in the so-called "sulphinic acid" portion of neoarsphenamine) can be found by oxidizing the neoarsphenamine in steps. From the results obtained we can tell whether the product will be toxic or nontoxic. The nearer the ratio of combined sulphoxalate to arsenic is to theory, the less toxic will be the product.

2 The structure of the molecule of neoarsphenamine, as it is at present accepted, does not express the real state of affairs. The new formula suggested, where the sulphoxalate group is attached directly to the arsenic and not to the amine group, is much more in agreement with experimental results.

3 The new facts presented should be an aid in the manufacture of a neoarsphenamine of low toxicity but high therapeutic value.

## HEMOAGGLUTINATION

## III HEMOAGGLUTINATION IN THE BLOOD OF CHICKENS\*

BY WARNER M. KARSHNER, B S, M D, F A C S, SEATTLE

IN FORMER papers of this series a brief statement was made covering work that has been done on hemoagglutination in the blood of animals. It is unnecessary to repeat that outline here. The technic therein described has been followed in the main and only new features will be noted in this paper.

## TECHNIC

I The chicken blood was secured from two widely different sources, namely (1) Meat shops where the birds were prepared for retail trade, and (2) from the thoroughbred White Leghorn strain of the Western Washington Experiment Station.

II The blood secured from public markets was often grossly contaminated, due, no doubt, to the nature of surroundings and the method of collection. It did not preserve well, even in the frigidaire, moreover, it was necessary to use 2 per cent sodium citrate normal salt solution to prevent coagulation. This necessitated repeated washings in normal salt solution to bring the cells to isotonicity.

## ISOAGGLUTINATION

Table I shows a series of sixty-four cross-typings of chicken blood. Of these sixty-four double typings, five only showed positive at both ends of the slide, fourteen showed positive at one end, forty-five double tests proved negative. In other words, of the one hundred twenty-eight separate tests, but twenty-four showed agglutination.

\*From the Bacteriology Department University of Washington Seattle.  
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TABLE I

RESULTS OF CROSS AGGLUTINATION OF CHICKEN RED CELLS WITH CHICKEN SERUM

SERUM X			SERUM Y		
O			O		
R	C	Y	R	C	X
			NO. CASES		
			PER CENT		
Both positive			8		
One positive			22		
Neither positive			70		
Total			100		

We note the absence of reactions that might be expected if the blood types of chickens were well developed and in comparatively large groupings. The fact that more agglutinations do not occur can be explained in two ways, namely, either the types in chickens are not clear and well developed, or the subnormal types are of relatively small percentage. The latter view, it would seem, harmonizes better with the known facts, since agglutination, when it does occur, usually appears prompt, vigorous and typical. Iso agglutination, moreover, as observed in the above series, appeared far less common among the thoroughbred White Leghorn flock than among the mixed strains and mongrels common in the open market. High percentages of positive agglutinations were also found when mixtures from these two sources were typed.

From a study of Table I it appears obvious that there are at least three distinct blood groups in chickens.

*Group A*—This constitutes by far the largest group. The red cells show no agglutination when mixed with serum from groups B or C.

*Group B*—This is a much smaller group. The red cells agglutinate when mixed with serum from Groups A and C.

*Group C*—This is the smallest group. The erythrocytes are not only clumped by the sera of Groups A and B but it also involves agglutination in the red cells of Group B.

It will be seen that Groups B and C are wholly incompatible, i.e., the sera and red cells mutually agglutinate each other.

## HETEROAGGLUTINATION

Heteroagglutination was defined in Parts I and II of this series of papers. Its presence in chicken blood is shown in a startling way in the following tables.

TABLE II

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH BOVINE SERUM

NO. CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
100	100	0	100	0

In this series of one hundred cases, the red cells of chicken blood were mixed with bovine serum. In not a single instance did heteroagglutination fail to appear promptly.

TABLE III

RESULTS OF AGGLUTINATION OF BOVINE RED CELLS WITH CHICKEN SERUM

NO CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
100	100	0	100	0

This table shows one hundred positive reactions when bovine red cells were mixed with chicken serum

TABLE IV

RESULTS OF AGGLUTINATION OF ADULT HUMAN RED CELLS WITH CHICKEN SERUM

NO CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
100	100	0	100	0

This shows one hundred cases where adult human red cells were mixed with chicken serum. In every instance agglutination occurred. The cells were clumped into large compact masses, larger and more firmly adherent than obtained when other sera were employed.

TABLE V

RESULTS OF AGGLUTINATION OF HUMAN UMBILICAL RED CELLS WITH CHICKEN SERUM

NO CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
101	101	0	100	0

This result is similar to the one in Table IV, except that the red cells came from the umbilical cord following delivery. In one hundred cases the erythrocytes gave a positive reaction. So far as could be observed, they were fully matured. This agrees with the findings in Part I, "Isoagglutination in the Blood of Infants."

TABLE VI

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH HUMAN UMBILICAL SERUM

SERUM TYPES	NO CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
II	12	6	6	50	50
IV	12	6	6	50	50

In this limited series chicken red cells were mixed with Types II and IV sera from human umbilical blood. Each type was represented by two different specimens, showing six tests, respectively. It will be seen that each specimen reacted consistently, when it caused agglutination in one instance, it did so in every instance, when it failed to react in one, it failed throughout the tests. These findings naturally suggest that the sera in the negative tests were immature and led to the following typings.

TABLE VII

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH TYPES II AND III SERA FROM ADULT HUMAN BLOOD

SERUM TYPES	NO CASES	AGGLUTINATION +	AGGLUTINATION -	PER CENT +	PER CENT -
II	104	0	104	0	100
III	104	88	16	85	15

Heteroagglutination, as shown by Table VII is indeed surprising. It shows 85 per cent positive reactions between Type III adult human serum and chicken erythrocytes, while Type II human serum when mixed with the chicken red cells did not show a single positive reaction in the whole series of 104 cases! This failure to react cannot be charged to defective Type II stock serum, since two separate allotments of sera were employed moreover they were simultaneously used with good results in typing both bovine and human umbilical bloods. Certainly, both allotments of Type II serum lacked the proper agglutinin necessary to clump chicken red cells but did not possess it for both human umbilical and bovine cells. Inquiry revealed the fact that both stocks of Type II sera used above were supplied by the same donor. It was assumed therefore that it was an individual characteristic and, to definitely ascertain the truth a large assortment of fresh human sera were secured.

TABLE VIII

RESULTS OF AGGLUTINATION OF CHICKEN RED CELLS WITH TYPES II, III AND IV ADULT HUMAN SERA

CHICKEN RED CELLS									
HUMAN SERA	TYPES	1	2	3	4	5	6	7	8
A	II	-	-	-	-	-	-	-	-
B	III	+	+	+	+	+	+	+	+
C	II	-	-	-	-	-	-	-	-
D	II	-	-	-	-	-	-	-	-
E	II	-	-	-	-	-	-	-	-
F	II	-	-	-	-	-	-	-	-
G	II	-	-	-	-	-	-	-	-
H	II	-	-	-	-	-	-	-	-
I	II	+	+	+	+	+	+	+	+
J	IV	-	-	-	-	-	-	-	-
K	IV	+	+	+	+	+	+	+	+
L	IV	+	+	+	+	+	+	+	+

In this series chicken red cells were mixed with twelve different human sera. A and B were the stock sera shown in the reactions of Table VII, and used here as controls. Of the ten remaining sera, seven gave negative results only. One Type II and two Type IV, gave only positive reactions. These results, condensed and retabulated, are shown in Table IX.

TABLE IX

RESULTS FROM TABLE VIII CONDENSED AND RETABULATED

SERUM TYPE	AGGLUTINATION +	AGGLUTINATION -	TOTAL	PER CENT +	PER CENT -
II	8	6	6	12 1/2	87 1/2
III	9	0	8	100	0
IV	16	8	24	66 2/3	33 1/3
Total	32	64	96	33 1/3	66 2/3

It will be seen from a study of this table that heteroagglutination resulting from a mixture of chicken red cells and human serum, is not a constant phenomenon. In fact, it is far less common than that obtained by mixing chicken red cells and bovine serum. Repeated washings of the chicken red cells in normal salt solution to remove all traces of chicken serum did not alter the findings; neither were the results materially affected by recourse to fresh unheated sera.

## CONCLUSIONS

- 1 Isoagglutination occurs occasionally in the blood of chickens
- 2 It appears more common among those of different breeds than among those of the same or similar strains
- 3 At least three types can be distinguished Type A, the largest group, that does not appear reactive Types B and C, two smaller groups, which show agglutination when the red cells are separately mixed with serum A These groups are incompatible, i e, when cross-typed they will agglutinate each other
- 4 Heteroagglutination resulting from a mixture of chicken and bovine bloods appears a constant phenomenon
- 5 Heteroagglutination resulting from a mixture of chicken serum and human red cells appears constant, with the blood elements inversely mixed, it often fails to appear

NOTE For references see Hemagglutination in the Blood, II, JOUR LAB AND CLIN MED, December, 1928

## STUDIES IN TOXICOLOGIC CHEMISTRY\*

### III THE REACTIONS OF THE PHENOLS AND PHENOLIC OR OPIUM ALKALOIDS WITH MARQUIS' REAGENT, THE NITRIC-ACID-FORMALDEHYDE REAGENTS, AND ERDMANN'S REAGENT

BY VICTOR E LEVINE AND CHARLES C FULTON, OMAHA, NEBRASKA

## INTRODUCTION

IN THE first article of this series Levine<sup>1</sup> showed that the phenol group is responsible for the reaction given by the opium alkaloids with Lafon's or Mecke's reagent, a solution of selenious acid in concentrated sulphuric acid In the second article Levine and Magiera<sup>2</sup> showed how the tests for morphine should be classified It was also shown in the latter article that the morphine reaction with Marquis' reagent (formaldehyde in concentrated sulphuric acid) belongs to the group of tests depending on the presence of the phenol group

Since then Fulton<sup>3</sup> in an independent article has announced two new reagents for the opium alkaloids These were called "Nitric-acid Formaldehyde Reagents," both being made from nitric acid, formaldehyde, and sulphuric acid One reagent differs from the other by dilution of its components They were distinguished as Reagents A and B To avoid unduly cumbersome names they will hereafter be referred to as the Fulton reagents

It seemed highly probable that these new reagents also react with the opium alkaloids because of the phenolic character of the latter If this were the case, it would be interesting to find certain nonalkaloidal phenols giving the same reaction as morphine or its allied compounds, or yielding characteristic reactions useful in identification With the foregoing ideas in mind, this present study was instituted

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It seemed best, in this connection, to repeat the tests with the Marquis reagent. In the previous study the time during which the reaction mixture was kept under observation was indefinite and extended in some cases to several days. In this study we have limited our observations to a period of about fifteen minutes. Laboratory air generally contains reactive substances, so that changes occurring very slowly or only after long standing may be of doubtful significance.

A survey of the reactions of Erdmann's reagent with opium alkaloids and phenols has also been included. This reagent, sulphuric acid containing a small quantity of nitric acid was originally introduced as a test for the opium alkaloids. We have found it to react with the phenols in general.

#### THE REAGENTS

The reagents investigated were made in the exact manner described.

##### FORMALDEHYDE-SULPHURIC ACID REAGENT (MARQUIS' REAGENT)

Two drops of USP formaldehyde solution (37 to 40 per cent) were added to 3 c c of concentrated sulphuric acid. The reagent deteriorates and should be prepared on the day used. Tested on brucine it should give no color.

##### NITRIC ACID SULPHURIC ACID REAGENT (ERDMANN'S REAGENT)

Four drops of concentrated nitric acid were mixed with 100 c c of distilled water. Five drops of this solution were added to 5 c c of pure concentrated sulphuric acid to form the Erdmann reagent.

##### NITRIC ACID FORMALDEHYDE SULPHURIC ACID REAGENTS (FULTON'S REAGENTS)

**Reagent A.** To one drop of concentrated nitric acid in a clean, dry test tube were added four or five drops of USP formaldehyde (37 to 40 per cent) and 5 c c of pure concentrated sulphuric acid. A violent reaction takes place on the addition of the sulphuric acid. When the effervescence subsides the reagent is ready for use. The reagent should be freshly prepared. If used throughout the day it becomes necessary to test it at intervals on known samples of morphine and codeine.

**Reagent B.** One drop of concentrated nitric acid in a clean, dry test tube was mixed with three drops of USP formaldehyde (37 to 40 per cent) and 1 c c of pure concentrated sulphuric acid. One c c of water and 4 c c of concentrated sulphuric acid were now added, and the mixture cooled by means of running water. The reagent should give the following reactions with known samples of morphine and codeine.

*Morphine*—brown (without any purple green, or blue) changing gradually to red.

*Codeine*—olive soon developing to deep bright blue, and gradually developing purple at least in the center of the solution.

The heat of mixing or the proportion of nitric acid to formaldehyde may have to be varied in order to produce a satisfactory reagent.

Reagent A is made with the components of the Marquis and of the Erdmann reagent. The nitric acid and the formaldehyde react violently, and the



formaldehyde is in excess. The reagent is therefore more nearly like Marquis' reagent than Erdmann's. However, the nitric acid does not lose the properties which it displays in Erdmann's reagent.

Since Reagent B is made from Reagent A by the addition of water, its characteristic reactions must be due to the same cause. It has the formaldehyde only very slightly in excess of the nitric acid. With some compounds it reacts much like a very sensitive Erdmann reagent. This is the case with morphine and codeine. Reagents A and B may be regarded as links connecting the Marquis and Erdmann reagents.

The Marquis reagent and the nitric-acid formaldehyde reagents are quite sensitive to phenols, and very small amounts of the latter suffice to give strong reactions. Erdmann's reagent is less sensitive, and larger amounts of phenols have to be employed in testing.

#### DESCRIPTIONS OF THE COLOR REACTIONS

It should be remembered that two people seldom describe the same color reaction in the same way. In making use of any reactions for the identification of an unknown, the analyst should always first try, if possible, the effect of the reagent on a known sample of the suspected substance.

In speaking of the various reactions we shall in the course of the paper refer to some of them as distinctive, characteristic, or specific. For the sake of clearness we shall define these terms in the sense that we employ them. By a *distinctive reaction* is meant one in which clear, bright, strong, and easily recognizable colors are obtained. By a *characteristic reaction* is meant one in which an easily recognizable color or succession of colors is obtained, and which is given by few, if any, other compounds which might be confused with the one for which the test is made. By a *specific reaction* is meant one given only by the compound for which the test is made and by no other substance so far as known.

#### REACTIONS OF THE OPIUM ALKALOIDS

The four reagents used were all introduced as giving useful color reactions for the identification of the opium alkaloids. Marquis' and Erdmann's reagents have been known for many years, but the nitric-acid formaldehyde reagents were introduced only last year. These reagents all react with the phenols in general.

The reactions with the opium alkaloids are given in Table I.

Erdmann's reagent is not of any great value.

The three reagents containing formaldehyde give distinctive reactions with morphine, codeine, oxydimorphine, apomorphine, papaverine, and narcotine. These alkaloids are readily distinguished from each other by means of these reactions. We shall later discuss the possibility of confusing them with other phenolic compounds.

With the four reagents heroine reacts like morphine and diomine like codeine.

#### REACTIONS OF THE PHENOLIC COMPOUNDS WITH THE FOUR REAGENTS

In Table II we report the detailed events following the application of the four reagents tested upon monophenols and their derivatives, diphenols

TABLE I  
ALKALOIDAL REACTIONS WITH THE FOUR TESTED REAGENTS

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Morphine and Heroin <sup>1</sup>	crimson gradually changing to purple then slowly to blue	momentary purple changing to green then to blue then to dirty violet red then slowly to green	brown changing to red	brown with tinge of orange
Codeine and Dionine	purple changing to blue purple	green changing to very dark green then slowly to dark blue then slowly to brown	olive, quickly changing to deep bright blue then gradually to purple blue	light yellow changing quickly to green then slowly to blue
Oxycodone	orange red changing to deep bright red	olive green changing to strong bright blue green	brown slowly changing to light blue green then to green	yellowish changing to dull green
Apomorphine	purple quickly changing to dark blue then gradually to dark green	purple changing to dull dark green then slowly brownish red purple develops	dirty green changing to blue then slowly to deep purple	dirty dull green changing gradually to deep red violet
Papaverine <sup>1</sup>	red purple develops slowly changing to red then slowly to brown red	deep bright blue soon develops tinge of green at center changing to deep blue throughout	light gray gradually changing to deep bright blue	negative
Narcotine <sup>2</sup>	purple soon changing to dirty brown gradually changing to yellow slowly to brown	dirty violet red changing to brown then to bright red	yellowish changing to bright carmine	salmon changing to orange yellow then gradually to orange, then slowly to red
Narceine <sup>3</sup>	orange develops changing to red then to brownish red	orange develops changing to red then to brown	yellowish brown with tinge of green then brownish solution purplish at edge changing to brown orange. The colors are poor	yellowish brown with green tint
Thebaine	Concentrated sulphuric acid alone and the reagents give brown red deep red orange on solution			
Cotarnine hydrochloride	Concentrated sulphuric acid and the formaldehyde reagents give light yellow			weak orange changing to orangish yellow at once

<sup>1</sup>In concentrated sulphuric acid alone the solid assumes a purple color and dissolves with a slight purple color

<sup>2</sup>In concentrated sulphuric acid alone a yellow color is formed possessing a faint tinge of green

<sup>3</sup>Concentrated sulphuric acid alone gives yellowish brown on solution brownish yellow gradually becoming somewhat orange

TABLE II

REACTIONS OF THE FOUR TESTED REAGENTS WITH PHENOLIC COMPOUNDS

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Phenol	momentary purple red changing to deep red insoluble, sticky mass	red brown changing to dark brown then to deep red	brown orange changing to dark brown then to dark green then slowly to dark red	light orange or yellow changing to light green then to light blue, slowly fades out
o Cresol (methyl phenol)	intense red, at first scarlet, later purplish	dark brown changing to red	brown changing to dark green, then gradually to dark red, beginning at the edge	yellow changing to light orange
m Cresol	momentary purple red changing to deep red in soluble, sticky mass	dark brown changing to deep scarlet	orange brown changing to dark brown then to deep red	yellow changing to green, slowly fades out
p Cresol	dark brown, in soluble	dark brown changing to olive brown	dark brown changing slowly to dark red	brown or orange brown
o Xylenol (4) (dimethyl phenol)	dark brown	dark brown	brown changing slowly to dark red	slight brown orange, changing to pink
m Xylenol (2)	orange changing to deep red	strong brown quickly changing to deep red	dark brown soon changing to dark red	brownish yellow changing to brown
m Xylenol (4)	brownish yellow changing to dark brown	dark brown	dark brown gradually changing to dark red	yellow changing to brownish orange then to pink
p Xylenol	yellow quickly changing to orange, then orange brown, then slowly to red	mahogany brown then to brown orange then soon to red	red solution with undissolved particles blue, on dissolving all red	orange brown changing to green fades slowly
Thymol	yellow changing to orange brown then to dark brown	brown changing to deep red	red solution with undissolved particles blue, on dissolving all red	yellow changing to bright green
Carvacrol	orange red changing to deep red	dark brown changing quickly to orange brown then gradually to deep red	brown develops quickly, gradually changing to deep red	yellow brown in soluble matter changing to light brown
Tyrosin (p hydroxy phenyl ethyl amine)	red brown develops quickly	strong dark brown	red brown changing to dull red, then gradually to brown	yellow
Tyrosin (p hydroxy phenyl alpha amino propionic acid)	light brown, changing to purple red	strikes dark brown dissolves light brown, develops dull red, changing to violet red	strikes dark red, dissolves dull red changing to deeper purple red	light yellow
alpha Naphthol <sup>1</sup>	undissolved solid becomes yellow brown, dissolving to olive green solution, changing quickly to yellow brown	olive green changing to olive brown, and then to brown gradually	green changing to blue green	undissolved solid becomes orange brown, dissolves to bright green solution soon fading to brownish yellow

<sup>1</sup>In concentrated sulphuric acid the solid strikes an orange color and finally dissolves to make a yellow solution

TABLE II—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
$\beta$ Naphthol	yellow orange, changing it once to olive green and then to dark green	olive brown changing to dull green and then to gray brown	greenish gray gradually changing to blue weak reaction	dirty green

*Monophenols With Halogen Group*

o Chlorophenol	purplish pink changing to deep purple red nt once insoluble	brown changing to red	brown changing to orange with some brown at center brown nt center becomes dark green then whole solution gradually changes to orange red	light yellow
m Chlorophenol	scarlet mostly in soluble matter nt first	brown changing to scarlet mostly in soluble matter nt first	brown changing to pink then to red	light yellow
p Chlorophenol	strong green changing to dull dark green in soluble matter	strikes brown purple dissolves brownish yellow soon changing to green	dull violet red a white scum soon forms on the surface of the solution	light yellow
Tribromo phenol	With concentrated sulphuric acid alone or the reagents a slight brownish coloration developed			
Thymol iodide	Scarcely soluble at first in concentrated sulphuric acid or the reagents but gradually changing to light brownish			

*Monophenols With Nitro Groups*

o Nitrophenol	With concentrated sulphuric acid alone or the reagents a yellow coloration developed			
m Nitrophenol	With concentrated sulphuric acid or with the reagents a yellow color developed			
p Nitrophenol	With concentrated sulphuric acid alone or the reagents slight yellowish color developed			
Trinitro phenol	negative	negative	negative	negative
1 Nitro 2 Naphthol	With concentrated sulphuric acid alone or the reagents a brownish yellow color developed			

*Monophenols With Amino Group*

p Amidophenols	dark blue	light gray blue changing to green	purple with tinge of black changing to dull blackish green	dark blue
Amidol* (1 3 diamino 4 hydroxy benzene dihydro chloride)	dark blue	purplish blue	black tinge changing to dull black purple	black violet
Metol (mono methyl p amido m cresol sulphate)	negative	yellow	light yellow	slightly yellow

\*In concentrated sulphuric acid the solid strikes red orange finally dissolving to make a yellow solution

\*In concentrated sulphuric acid a dark blue color is formed

In concentrated sulphuric acid a dark blue color is produced

TABLE II—CONT'D

*Monophenols With Nitro and Amino Groups*

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Picramic acid (dinitro amino phenol)	With concentrated sulphuric acid alone or with the reagents, a light brown color is produced			

*Ethers of Monophenols*

Anisol (methyl phenyl ether)	pink changing to deep dark red	purplish red	brown changing to olive green, soon to red	brownish orange tint changing to green then to pink
Anethols (p propenyl anisol)	yellow brown	yellow brown	brown, changing to dirty violaceous brown then to black	orange red
Phenetol (ethyl phenyl ether)	pink changing to deep bright red	violet red gradually changing to deep red	pink and brown changing to red and olive brown, then to deep ear mine red	faint yellow changing to light green
Phenacetin (acetyl derivative of p amino phenetol)	gradually very faint brownish yellow	faint purple changing to purplish green changing to faint brownish, reaction very weak	faint lavender, very, very weak reaction	faint yellow
Thyroxin (tetra iodo p hydroxy phenyl ether of tyrosine)	brown	orange brown	negative	negative

*Nitrated Ethers of Monophenols*

m Nitro phenetol	With concentrated sulphuric acid alone or with the reagents, a yellow orange coloration developed			
p Nitro phenetol	With concentrated sulphuric acid alone or with the reagents a yellow color developed			

*Monophenols With Alcohol Group*

Diathesin <sup>6</sup> (o hydroxy benzyl alcohol)	insoluble, red changing to violet	insoluble, purple	insoluble particles red violet, solution becoming light pink	insoluble, particles dark red
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*Monophenols With Aldehyde Group*

p Hydroxybenzaldehyde	negative	negative	negative	negative
Anisic aldehyde (p methoxy benzaldehyde)	With concentrated sulphuric acid alone or with the reagents a weak yellow brown developed			
Salicylic aldehyde (o hydroxy benzaldehyde)	Concentrated sulphuric acid alone or the reagents produce a brown yellow color, changing at once to deep orange red			

<sup>6</sup>In concentrated sulphuric acid alone an orange-red insoluble sticky mass is formed

<sup>6</sup>In concentrated sulphuric acid alone insoluble particles turn red and solution turns pink

TABLE II—Cont'd

*Monophenols With Carboxyl Group*

	MARQUIS REAGENT	REAGENT A	REAGENT D	ERDMANN'S REAGENT
Salicylic acid (o hydroxy benzoic acid)	slowly develops red	gradually develops pink changing to red	gradually develops salmon pink changing to orange	negative
Aspirin (acetyl salicylic acid)	slowly develops red	gradually develops pink, changing to red	gradually develops salmon pink, changing to orange	negative
m Hydroxy benzoic acid	light yellow	faint green	faint green	faint yellow
p Hydroxy benzoic acid	faint orange	faint salmon orange develops	faint orange develops	negative
b Oxynaphthoic acid <sup>†</sup>	soon becomes green	brown changing to dark olive green which soon changes to dark olive	brown changing rather slowly to olive	negative

*Monophenolic Ethers With Carboxyl Group*

Anisic acid (p methoxy benzoic acid)	With concentrated sulphuric acid alone or with the reagents, a slight blue green color developed			
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*Esters and Salts of Phenolic Acids*

Methyl salicylate	slowly develops red	gradually develops pink changing to red	gradually develops salmon pink changing to orange	negative
Salol (methyl salicylate)	red soon develops	brown orange changing at once to red	develops salmon pink gradually changing to orange	light yellow changing to light green
Salphosalicylic acid	slowly develops red	gradually develops pink changing to red	gradually develops salmon pink changing to orange	negative
Sodium salicylate	slowly develops red	gradually develops pink changing to red	gradually develops salmon pink changing to orange	negative
Sodium or zinc phenolsulphonate	salmon orange changing to deep orange red	brownish orange gradually changing to orange-red	gradually develops orange that changing to orange red	negative

*Diphenols*

Pyrocatechin (o dihydroxy benzene)	dirty red changing to dirty purple	dark brown changing to brownish violet then to dark brown	dark brown changing to dark brownish red with blue edge above level of solution	brown changing to olive then to olive green
Adrenalin <sup>s</sup> (o Dihydroxy phenol hydroxy ethyl methyl amine)	becomes brown	strikes dark brown dissolves orange brown	orange brown	yellow

<sup>†</sup>With concentrated sulphuric acid alone, yellow color formed<sup>s</sup>In sulphuric acid alone the solid particles strike a red color and finally form a light yellow solution

TABLE II—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Resorcin (m dihydroxy benzene)	yellow changing to orange yellow solution with scarlet flecks, gradually orange solution	brown changing to scarlet with deeper red flecks	brown changing to dirty purple then deep blue, then solution red at edge, blue at center	yellow, violet develops and changes to strong violet blue
Orcinol (methyl resorcinol)	yellow changing to orange brown	yellow brown changing to orange brown	brown changing to brown red	yellow or brown yellow
Hydroquinone (p dihydroxy benzene)	brown	bright orange soon changing to brown orange, then gradually to orange red	brownish purple changing to brown red	brownish yellow

*Ethers of Diphenols*

Guaiacol <sup>9</sup> (monomethyl ether of pyrocatechol)	deep red changing to dull red violet	dark brown changing to dark purple	dark brown quickly changing to dark red	strikes green brown, dissolves brown
Guaiacol carbonate	pink changing to carmine	rose red changing to bright red	develops pink	negative
Eugenol <sup>10</sup> (allyl 3, 4, guaiacol)	becomes dark red changing gradually to brown then to dull violet red	dark brown changing to dark purple red then dark brown	dark brown changing to dark brown red	strikes brown yellow, dissolves deep orange red

*Ethers of Diphenols With Alcohol Group*

Vanillyl <sup>11</sup> alcohol (m methoxy p hydroxy benzyl alcohol)	dark red changing to dirty dull violet	purple changing to dark blue then to black	dirty dull violet	red changing to dull red violet
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*Ethers of Diphenols With Aldehyde Group*

Vanillin m methoxy p hydroxy benzaldehyde	With concentrated sulphuric acid alone or with reagents a light yellow color developed			
Papieronal (heliotropin anhydride of vanillin)	With concentrated sulphuric acid alone or with reagents a yellow color developed			
Veratraldehyde	With concentrated sulphuric acid alone or with reagents a yellow color developed			

*Ethers of Diphenols With Carboxyl Group*

Vanillic acid (m methoxy p hydroxy benzoic acid)	yellow orange changing to orange red	brown yellow changing to green with yellow border	yellow brown develops gradually	yellow
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<sup>9</sup>In concentrated sulphuric acid alone a brown color appears<sup>10</sup>In concentrated sulphuric acid alone a deep orange red is formed<sup>11</sup>In concentrated sulphuric acid alone a deep carmine red is formed

TABLE II—CONT'D

*Triphenols*

	MARQUIS' REAGENT	FEAGENT A	FEAGENT B	ERDMANN'S REAGENT
Pyrogallol (1, 2, 3 trihydroxy, benzene)	bright orange red changing to dark red brown	dark brown changing brownish red	dark brown changing to reddish brown	reddish changing at once to brown, then gradually to dull green
Phloroglucin (1, 3, 5, trihydroxy benzene)	bright orange changing to scarlet	yellow changing to orange red	particles red gradually dissolves to deep purplish red	slightly yellow

*Triphenols With Carboxyl Group*

Gallie acid (3, 4, 5, trihydroxy benzene)	light brown orange changing to green	brown changing to dull green gradually changing to dark red	olive green changing to brown	yellow brownish
Tannic acid (digallic acid)	orange brown changing to dark brown insoluble particles poor reaction	dark brown in soluble particles poor reaction	dark brown in soluble particles poor reaction	pink brown changing to brown, poor reaction

*Salts of Triphenolic Acids*

Dermatol (bismuth subgallate)	light yellow	orange red changing gradually to brown orange	orange changing gradually to red	light yellow brown changing to light brown
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*Glycosides Yielding Phenols on Hydrolysis*

Arbutin (yields hydroquinone on hydrolysis)	brown	brown changing to brownish orange	deep purple changing to brown quickly	yellow changing to brownish yellow
Salicin (yields on hydrolysis saligenin or o hydroxy benzyl alcohol)	strong red changing to dark dull red	deep red	bright scarlet	orange red
Phloridzin <sup>14</sup> (yields on hydrolysis the phloroglucin ester of p hydroxy atropic acid)	yellow changing to brown	brown	red brown changing to orange brown	light yellow
Aesculin (yields on hydrolysis aesculetine or 4, 5 dihydroxy coumarin)	With concentrated sulphuric acid alone or with the reagents a faint yellow color develops			

*Miscellaneous Compounds*

Brucine	negative	strikes red dissolves without color orange develops changing to yellow	strikes red dissolves orange changing to yellow	strikes red dissolves orange changing to yellow
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<sup>14</sup>In concentrated sulphuric acid a brownish yellow color develops<sup>15</sup>In concentrated sulphuric acid alone a red color is produced<sup>16</sup>In concentrated sulphuric acid alone a light yellow color develops which changes to light orange



TABLE II—CONT'D  
MISCELLANEOUS COMPOUNDS—CONT'D

	MARQUIS' REAGENT	REAGENT A	REAGENT B	ERDMANN'S REAGENT
Diphenyl amine <sup>15</sup>	negative	intense deep blue	intense deep blue	deep blue
$\alpha$ Naphthyl amine	green or blue green develops	brownish changing to dirty dark green, then to dark brown, then to dark brown red with violet tint	solution gradually becomes light brown with lavender edge, changing to purple red, then slowly to deep red	faint yellow, fading
$\beta$ Naphthyl amine	deep blue develops, quickly changing to blue green	beautiful blue soon develops, changing to deep blue green gradually	yellow brown develops gradually	faint yellow
Benzidine	With concentrated sulphuric acid alone or with reagents faint purple changing to slight red developed			
Phenyl hydrazine hydrochloride	orange soon develops	orange yellow develops, weak reaction	becomes slightly greenish	yellow
Tryptophane <sup>16</sup> ( $\beta$ indol $\alpha$ amino propionic acid)	brown	light brown	yellow brown, changing to orange	yellow
Ephedrine	negative	negative	negative	negative

<sup>15</sup>In concentrated sulphuric acid alone a light dull green develops

<sup>16</sup>The reactions recorded are very weak

and their derivatives, triphenols, and phenolic glucosides. A brief discussion of the results obtained follows.

The four reagents react with phenols, ethers of phenols, and other phenolic compounds.

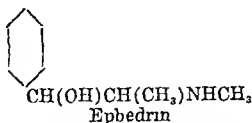
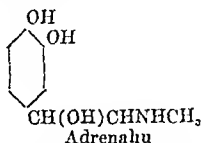
The Marquis reagent is useful for phenols in general. Most of them give red or brown colors.

Erdmann's reagent gives comparatively weak reactions. It may, however, be used to very great advantage with resorcin, and is also of some use with thymol and various other phenols.

Fulton's Reagent A gives distinctive and striking reactions with the opium alkaloids, quite different from those given by Marquis' reagent. With most phenols, however, it gives reactions similar to those with the Marquis reagent, and where this is the case it has no advantage over the latter. Reagent A is easily prepared.

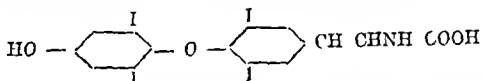
Fulton's Reagent B requires a little practice to prepare properly, and has to be tested on known morphine and codeine (or on a known sample of the substance for which the test is made) before it can be used. In many cases, however, this reagent will be well worth the trouble of preparation. It is useful for the opium alkaloids, especially in testing for codeine or diionine. With the majority of phenols the reactions are very similar to those with the Marquis reagent or Reagent A. Distinctive and characteristic reactions, however, are obtained with phenol, p-cylenol,  $\alpha$ -naphthol,  $\beta$ -naphthol, resorcin, and pyrocatechin.

Adrenalin is a diphenol. Ephedrin behaves physiologically like adrenalin, but it has no phenolic groups within its molecule. The formulas for the two compounds are as follows:



The similarity in the composition of the side chains in these two compounds indicates the common factor responsible for their resemblance in physiologic behavior. From our standpoint it is interesting to note that the nonphenolic compound, ephedrin gives negative reactions with our four reagents while adrenalin a diphenol gives positive reactions. The four reagents can, therefore be employed for the chemical differentiation of adrenalin and ephedrin.

Thyroxin, according to E. C. Kendall,<sup>4</sup> was believed to be a compound derived from tryptophan. Harrington<sup>5</sup> however, has recently proved that the thyroid hormone is derived from the phenolic amino acid, tyrosin. According to his researches thyroxin is a tetra iodo derivative of the p hydroxy phenyl ether of tyrosin. The formula of this phenolic ether is given below:



The positions of the iodine atoms in this ether molecule have been determined by Harrington Barger.<sup>6</sup> The iodine atoms occupy the 3,5,3',5' positions. It is interesting to note that the positive reactions we obtained with thyroxin with the Marquis reagent and with Fulton's Reagent A harmonize with Harrington's findings in reference to the phenolic character of this hormone.

The two naphthylamines give good reactions with Reagents A and B. Since the color responses are different, either or both of the reagents can be used to differentiate the alpha compound from the beta compound.

The presence of the aldehyde group in the phenol compounds is the cause of negative reactions with all the four reagents. This has been the experience with para hydroxybenzaldehyde, para methoxy benzaldehyde (anisic aldehyde), o hydroxy benzaldehyde (salicylic aldehyde), with vanillin with piperonal, and with veratraldehyde.

The presence of the nitro group is also inimical to the formation of a color reaction with any of the four reagents. Negative results have been obtained with o nitrophenol, m nitrophenol, p nitrophenol, trinitrophenol, di nitro amino phenol, m nitro phenol, and p nitro phenol.

A comparatively large number of halogen atoms in the molecule render the reagents inert. Tribromophenol and diiodothymol (thymol iodide) do not respond to the four reagents. On the other hand, o chlorophenol, p chlorophenol, and m chlorophenol have been found to be reactive.

## TYPES OF REACTIONS

A great variety of effects are obtained with the four reagents

With morphine and with codeine each of the reagents give a different reaction, although the reaction of Reagent B somewhat suggests a very sensitive Erdmann reagent. Reagent A gives a reaction entirely different from that of either the Marquis reagent or Reagent B.

With oxydimorphine the reactions of Reagents A and B are similar, and bear a general resemblance to the Erdmann reaction, but all are entirely different from the reaction with Marquis reagent. In this case it will be found that adding any oxidizing agent along with the Marquis reagent, such as a small crystal of potassium permanganate or chromate, will change the reaction entirely and make it similar to that of Reagent A. This is not the case with morphine or codeine.

With *m*-cresol, and with many other phenols, including *m*-xylenol (2), carvacrol, veratrol, and phenetol, the reactions of the three reagents containing formaldehyde are very similar, while Erdmann's reagent also reacts. The colors with the formaldehyde reagents are usually red or brown.

With resorcin, and with a number of phenols, including *p*-xylenol, thymol, phenol and anisol, the reaction of Reagent A is very similar to that of Marquis' reagent, and usually less distinctive, while the reaction of Reagent B is decidedly different, and the most distinctive of the four.

Salicylic acid and similar compounds do not react with Erdmann's reagent, and the reactions with Reagents A and B are weaker than with the Marquis' reagent. Brucine and diphenylamine do not react with Marquis' reagent, but with Reagents A and B give reactions similar to those with Erdmann's reagent.

## POSSIBILITY OF MISTAKING A PHENOL FOR AN OPIUM ALKALOID

With Marquis' reagent most of the opium alkaloids give purple colors, while most of the phenols give red or brown. However, some phenols do give purple colors.

A number of phenols have been found which give with Marquis' reagent reactions more or less like those of morphine. These include pyrocatechin, guaiacol, vanillyl alcohol, and veratrol. These substances, however, all give precipitates on diluting the reaction-mixture with water, while morphine and codeine give clear yellow solutions. We have not confirmed the previous observation that *p*-cresol also reacts like morphine.

Reagent A gives a very valuable specific reaction with morphine. The reactions of phenols in general are quite different. It is probable that no compound exists which gives the same reaction as morphine with this reagent, save, of course, such closely related derivatives of morphine itself as heroine. Only one compound,  $\beta$ -naphthol, has been found as yet which gives with Reagent A a reaction at all similar to that of codeine.

With Reagent B codeine gives a distinctive and characteristic reaction. Again  $\beta$ -naphthol shows a certain resemblance, while di-phenylamine might

easily be mistaken for codeine so far as this one reaction is concerned Reagent B is not of much value for morphine

The three reagents containing formaldehyde identify not only morphine and heroine codeine and diuine but also oxydimorphine, apomorphine, papaverine, and nareotine By considering the reactions of these opium alkaloids with the three reagents it should be possible to identify each of them beyond a reasonable doubt

#### SUMMARY

The Marquis reagent, the Erdmann reagent and the Fulton reagents react with phenols, ethers of phenols phenolic alkaloids and other phenolic compounds They can be applied in a practical way to identify these compounds

These reagents are not absolutely specific for phenols, since certain non phenolic compounds  $\alpha$  and  $\beta$  naphthylamine phenylhydrazine and tryptophan, also yield reactions

The presence of the aldehyde group in the phenol compound inhibits the color reactions obtained with the four reagents

The presence of the nitro group is also inimical to the formation of a color reaction

Tribromophenol and diiodothymol do not respond to the four reagents but the monochlorophenols have been found to be reactive

The four reagents can be utilized to differentiate the opium alkaloids, to distinguish the phenolic hormone adrenalin from the nonphenolic compound ephedrin to distinguish tyrosin from the nonphenolic amino acids, and to differentiate alpha naphthylamine from the beta form

The three reagents containing formaldehyde are the most valuable Erdmann's reagent gives comparatively weak reactions

Some of the phenols give some reactions similar to those of morphine codeine or other opium alkaloids However by using the three formaldehyde reagents, or all four reagents, it should be possible to identify morphine codeine, oxydimorphine apomorphine, papaverine, and nareotine beyond a reasonable doubt

#### REFERENCES

- <sup>1</sup>Levine, V E Studies in Toxicologic Chemistry I The Detection of the Opium Alkaloids by Selenious Sulphuric Acid the Specificity of This Reagent for the Phenolic Group JOUR LAB AND CLIN MED, 1926 xi 809
- <sup>2</sup>Levine, V E, and Maguarn, E A Studies in Toxicologic Chemistry II The Formaldehyde Sulphuric Acid Reaction of the Opium Alkaloids JOUR. LAB AND CLIN MED, 1927, xii, 773
- <sup>3</sup>Fulton, C C Some New and Improved Tests for Morphine and Related Alkaloids JOUR LAB AND CLIN MED 1928 xiii 750
- <sup>4</sup>Kendall E C Isolation of the Iodine Compound Which Occurs in the Thyroid Jour Biol Chem, 1919, xxxix 125  
Kendall E C, and Osterberg A E The Chemical Identification of Thyroxin, Jour Biol Chem, 1919, xl, 265
- <sup>5</sup>Harington C R Chemistry of Thyroxin I Isolation of Thyroxin From the Thyroid Gland Biochem Jour, 1926 xx 293
- <sup>6</sup>Harington C R Chemistry of Thyroxin II Constitution and Synthesis of Desiodo Thyroxin, Biochem Jour, 1926 xx 300
- <sup>7</sup>Harington C R, and Barger G Chemistry of Thyroxin III Constitution and Synthesis of Thyroxin, Biochem Jour, 1927, xxi, 169

# LABORATORY METHODS

## NEW METHOD FOR RECORDING MINUTE FLUCTUATIONS OF FLUIDS IN CAPILLARY TUBES WITHOUT A FLOATER\*

BY MAURICE MUSCHAT, M D , PHILADELPHIA, PA

RECORDING fluctuations of liquids becomes quite a problem when the manometer is a capillary tube and the excursions of the moving fluid level are small. A floater cannot be used in such cases.

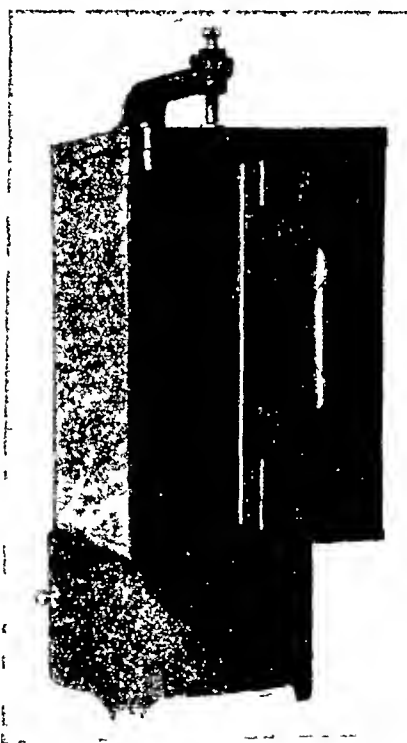


Fig 1 —Complete outfit of the recording camera

While working on the contractions of the milking muscle of the kidney, the necessity arose for obtaining a permanent record of the up and down movements of the fluid in a capillary, and we therefore devised the following apparatus.

An ordinary revolving drum was used. A metal box with a removable lid was constructed, measuring in width and height 2 cm more than the re-

\*From the Department of Research Surgery, University of Pennsylvania.  
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volving drum. Two openings were made, one in the center of the bottom, the other in the middle of the lid, in order to permit the central axial rod of the drum to carry the cylinder inside of the metal box. On the frontal side of the box a slit, extending all the way down was made with a lateral sliding bar to make the slit as wide as desired. A metal strip slides over the slit and completely darkens the inside of the box. (Fig 1)

The box with the revolving drum inside of it fits into the main drum stand by means of the lower end of the axial rod which falls into the slot, and the upper end which fits into the arm of the stand and is fastened by means of a screw. (Fig 2)

The box containing the revolving drum is easily removed from the stand and carried into a dark room where it is loaded. A piece of bromsilver paper

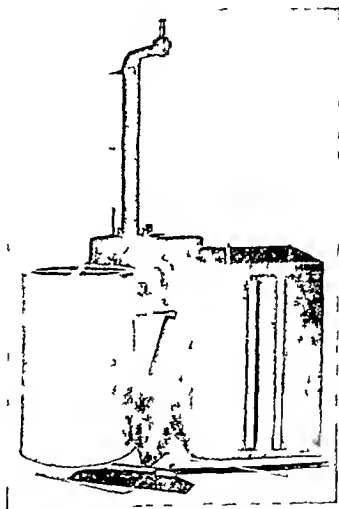


Fig. —Recording camera taken apart to show the various parts

having been cut to the size of the drum is now put around the removed drum and held in place by two rubber bands. The drum is then replaced in the box by sliding it over the axial rod. After having reached the bottom of the box, the drum is brought up for about 2 cm and the upper holding screw tightened. The lid is then fit over the top and the outer screws tightened. The box can now be taken into the light and put back on the stand ready for use. Having thus constructed the receiving camera, the problem was to have something on top of the moving fluid in the capillary in order to get a white or black line on the recording paper.

Capillaries with a diameter of 0.5 mm are entirely too small to carry any kind of a floater, even a small piece of dark glass sticks to the walls of

the tube. A drop of a yellow liquid lighter than water and insoluble in water floating on top of the moving liquid of the capillary would answer the question. At a suggestion by Dr. E. Wagner of the Department of Chemistry these requirements were met by using a solution of azo-benzene, a dark yellow organic substance, dissolved in benzene. This solution does not mix with water, and being reddish-yellow in color excludes the penetration of any light through the floating drop.

It is also worth noting that the azo-benzene itself is entirely insoluble in water, which gives the assurance of the yellow drop not mixing with the important oxygenated physiologic solutions used in the experiment and in this way eliminates the possible interference with the actual preparation. The solu-

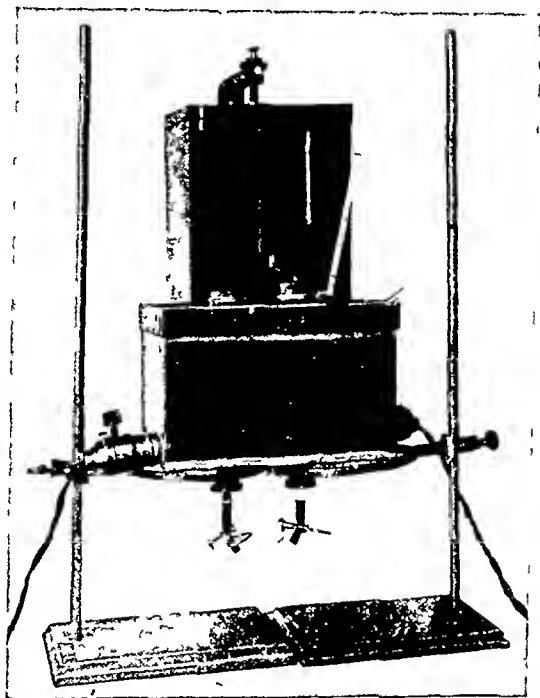


Fig. 3—Recording camera in action in conjunction with a constant temperature bath

tion of azo-benzene in benzene is aspirated into very fine capillaries which fit into the manometer tube. Having sealed one end, these loaded capillaries are kept ready for use.

After having prepared the main part of the experiment and being ready to record the obtained fluctuations, one of the small capillaries with the yellow liquid is inserted into the upper end of the manometer tube and brought down until it reaches the fluid level, the upper end of the capillary is then broken off and the yellow liquid is permitted to run out on top of the manometer fluid. In order to obtain a good record, it is best to put in a yellow column of 1 cm. in height.

The capillary manometer is now brought close to the recording box and pushed into the frontal slit (Fig. 3). Black paper strips are being used to

cover the upper and lower parts of the slit, where the penetration of light is not desirable. A piece of metal strip with a 0.5 mm slit slides in front of the manometer to reduce the light and to obtain a clear picture. A 60 watt frozen bulb is used facing the box at a distance of 30 cm. The opening of the



Fig 4 —Chart showing recorded fluctuations that were hardly noticeable with naked eye

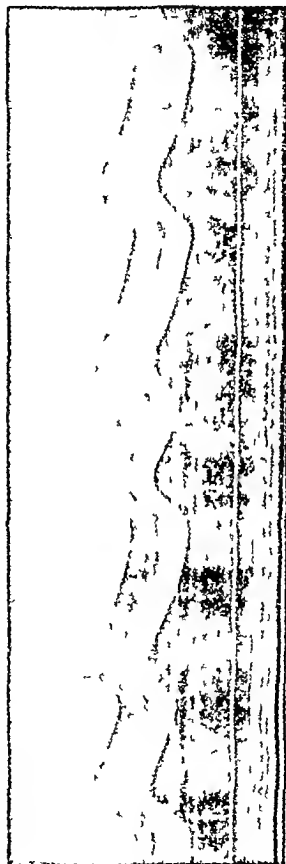


Fig 5 —Chart showing heavy fluctuations recorded by the camera.

lamp shade is covered with a piece of pasteboard with a central opening of about 3 cm in diameter.

After the capillary manometric tube has been set into the slit of the box the proper screening accomplished the light turned on and focused, the drum



is made to run as slowly as desired, recording every movement of the yellow diop in the capillary. It is obvious that movements in several tubes could easily be recorded in this way simultaneously.

Two records are appended (Figs 4 and 5), one where the fluctuations were hardly noticeable, the second where stronger movements were seen. The time can easily be recorded either by turning out the light or by placing something in front of the slit every minute.

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## AN APPARATUS FOR AUTOMATICALLY ELICITING AND RECORDING THE ACHILLES JERK\*

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BY W. W. TUTTLE, PH. D., IOWA CITY, IOWA

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THE susceptibility of the deep reflexes to changes in the neuromuscular mechanism has brought forth a vast amount of research dealing with the factors responsible for these changes. Up to the present time most of the quantitative investigations have been based upon the knee jerk of both animal and human subjects. It has been demonstrated in this laboratory that the Achilles jerk is just as reliable as an index to neuromuscular changes as is the knee jerk. Furthermore there are a number of advantages in favor of using the Achilles jerk.

For the quantitative study of the Achilles jerk an apparatus has been devised using principles identical to those involved in the apparatus developed for the eliciting and recording the knee jerk (Tuttle, 1924).

The apparatus consists of the following three parts: (1) the stimulating unit, (2) the subject unit, (3) the recording unit.

*The Stimulating Unit*—The details of this unit are shown in Figs 1 and 2. Essentially it consists of a motor, gear-box, tripping device and stimulating hammer mounted on an adjustable stand. The speed of a 1750 R P M motor is reduced to 10 R P M by means of a worm gear. This reduction necessitates a ratio of 1:175 between the number of teeth in the worm and the number in the gear. To reduce the noise the worm gear runs in grease. A wooden wheel 8 inches in diameter is attached to the shaft of the gear. The stimulating hammer is mounted in bearings so that the hammer handle extends past the pin placed near the edge of the wooden wheel. As the wheel turns the pin engages the hammer handle, lifts the hammer, dropping it when the pin ceases to engage the handle. A stop supports the hammer when the machine is not in motion.

The apparatus is flexible with regard to both rate and strength of stimuli. The hammer handle is provided with a weight which serves to vary the strength of the stimuli as its position with relation to the fulcrum is changed. An increase in the rate of stimulation is brought about by adding a pin to the tripping wheel. If an additional pin is placed exactly opposite the one already

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\*From the Department of Physiology, State University of Iowa, Iowa City.  
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in the wheel the rate of stimulation is uniformly doubled. A rheostat in the motor circuit serves to control the speed of the motor. By adjusting this rheostat one may secure any desired speed slower than 10 R P M.

*The Subject Unit*—Although detailed drawings of this unit are not included, the arrangement of the leg and foot for the delivery of the stimuli to the Achilles tendon is shown in Figs 1 and 2. The subject is placed in a prone position on a well padded table. A support is fastened to the table to hold the leg and foot secure. In order that the member involved may be free to move, the observer is placed so that his foot extends well over the edge of the table. This position is also advantageous since it is at once suitable for the application of the stimuli and the recording of the response. The stimulating

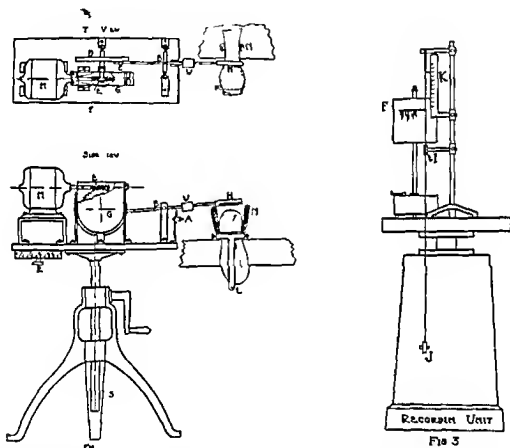


Fig 1—A Hammer support B fulcrum of the hammer L worm gear G gear box H hammer L T clamp

Fig 2—M Motor V leg support P Rheostat S adjustable stand W weight

Fig 3—F Kymograph I guide pulley J guide pulley K Iron stand

unit is placed in proper proximity to the Achilles tendon and adjusted to the position suitable for the experiment.

*The Recording Unit*—This unit is set at the subject's head. Its details are shown in Fig 3. The record is made by attaching the toe of the shoe to a recording stylus as shown in Figs 1 and 2. A T-clamp is fastened to the sole of the shoe close to the heel. Stout fish line runs from a hole in the T-clamp under the table through guide pulleys to a rubber band suspended from an iron stand.

The importance of uniform tension on the toe is easily demonstrated by varying this factor. It is evident that within limits an increase in tension increases the extent of the reflex. In order that such a determinant may be controlled a pointer is attached to the line and a millimeter scale is fastened to

the non stand By adopting a uniform position for the pointer on the scale one controls the factor of tension.

The record is made on a slowly moving kymograph equipped with a clock release When the jerk is elicited, the recording stylus is drawn down making a record comparable to the one shown in Fig 3 When the toe returns to its original position, the elasticity of the rubber band pulls the stylus back to the base-line Since the base-line is at the top, the records are read from right to left

The displacement of the toe represented by vertical lines on the record furnishes an index to the extent of the reflexes A mathematical index is established by finding the average in millimeters of a given number of responses For all practical purposes ten stimuli per minute are delivered over a period of ten minutes This gives a fair sampling of the responses of a subject

#### SUMMARY

An apparatus has been devised for automatically eliciting and recording the Achilles jerk It delivers uniform stimuli at a constant rate to the Achilles tendon The displacement of the toe of the foot recorded as vertical lines on a slowly moving kymograph furnishes the index to the extent of the reflex A mathematical index is established by finding the average height in millimeters of a given number of responses

#### REFERENCE

Tuttle, W W Am J Physiol 68 38, 1924

### A NOTE ON THE STANDARDIZATION OF ANTIGEN FOR USE IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS\*

By A. SCHABILE, A B, ALBANY, N Y

**D**URING the past year or more, in standardizing antigens for use in the complement-fixation test for syphilis, considerable difficulty has been experienced in preparing successive cholesterolized extracts of uniform sensitivity by a method similar to that of Neymann and Gager<sup>1</sup> Beef-heart tissue, which was used as the basis for these antigens, appears to vary in the lipid content with the result that two antigens prepared by the same method from different lots of beef hearts frequently varied in sensitivity Several successive antigens prepared by the same method were all less sensitive than the standard antigen As it was thought that possibly the ether extraction had been somewhat excessive, in one or two subsequent antigens, a less thorough ether extraction was employed without, however, improving the quality of the resulting antigen An attempt was made to bring one of these less sensitive antigens up to standard

\*From the Division of Laboratories and Research New York State Department of Health Albany

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by increasing the percentage of cholesterol with which it was reinforced, and it was found that quite marked increase in the cholesterol content had little effect in increasing its sensitivity.

In view of the possibility that the lack of sensitivity of these antigens was due to overconcentration rather than underconcentration of lipoids, one of the less sensitive antigens was diluted with an equal part of absolute alcohol and then reinforced with the 0.4 per cent cholesterol routinely employed. The ordinary dilutions in salt solution were made and the antigen was compared with the standard in a large series of tests with gratifying results. Of five hundred and sixty comparative tests there was approximate agreement in the results obtained with the two antigens in 96.6 per cent.

When several of the other less sensitive antigens were treated in the same way, the sensitivity of one was increased to that of the standard antigen by the addition of an equal part of alcohol. Another required one and one half parts of alcohol to bring it up to the standard and when two parts were added, it became more sensitive. Yet, when the standard antigen was diluted with an equal part of alcohol its sensitivity remained unchanged. Thus, it appears that in some antigens the optimum concentration of lipoids covers quite a wide range. In all, five antigens diluted with alcohol have now been compared with the standard in a large series of tests and their sensitivity has been successfully adjusted by the addition of from one to one and one half parts of alcohol.

The increase in sensitivity of the alcohol-diluted antigens appeared to be due, to some extent, to an increased instability of the final dilution in salt solution, as the dilutions of these antigens tended to become anticomplementary after they had stood for several hours. The increase in anticomplementary effect was more marked with some pools of guinea pig complement than with others. The dilutions were always satisfactory for as long as two hours and in most instances considerably longer. It is wise therefore to prepare a fresh dilution if any tests are to be made after the antigen dilution has been standing for a period of more than two hours. It is also advisable to give all antigen dilutions a period of approximately fifteen minutes after dilution with salt solution to reach a state of colloidal equilibrium.

This method of increasing the sensitivity of an antigen by dilution with alcohol, which has been described in connection with the precipitation test, seems of sufficient importance to justify publication of its application in standardizing antigen for use in the complement fixation test.

#### REFERENCES

- 1 Neymann C. A., and Gager L. T. A New Method for Making Wassermann Antigens From Normal Heart Tissue. *J. Immunol.* 2: 113, 1914.
- 2 Kahn R. L., Nagle N., and Kendrick P. L. Studies on Antigen for the Kahn Test. Uniformity in Sensitiveness of Standard Antigen. *J. Infect. Dis.* 41: 111, 1927.

## A COMPARISON OF THE RESULTS OF MEINICKE, KAHN, AND WASSERMANN REACTIONS\*

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AMONG the numerous precipitation tests used for the serodiagnosis of syphilis, the Meinicke turbidity reaction has been well recommended on account of its reliability and simplicity. This test has had a widespread use in Europe and is apparently gaining favor in tropical countries. In the literature of this country, however, there are only a few reports of the results of comparative tests with this procedure and the complement fixation and flocculation tests used to obtain serologic evidence of syphilis. This paper adds to the information on that point by presenting the results of a series of parallel tests with the Wassermann, Kahn, and Meinicke reactions on 1000 specimens of blood serum.

In the course of its development, the Meinicke test has undergone a number of modifications. Of these, the one which is now coming into more general use is known as the TR or turbidity reaction. It is described by Meinicke<sup>1</sup> in a paper published in 1927 in the *JOURNAL OF LABORATORY AND CLINICAL MEDICINE*. This article deals with the development of the test, and gives a bibliography of papers reporting experiences with it in attempts to evaluate its simplicity, specificity, and sensitiveness.

Among the recent articles not cited by Meinicke, we have found only three which report a comparison of the Wassermann, Meinicke, and Kahn tests.

Ruediger<sup>2</sup> reported examining 311 consecutive specimens with his modification of the Wassermann test, the Kahn test, and the Meinicke test with about 87 per cent agreement between the three tests. In a second paper, Ruediger<sup>3</sup> reported the examination of 265 consecutive specimens with a Kolmer-Wassermann test, Ruediger-Wassermann test, the Kahn test, and Meinicke test. There was 84.5 per cent agreement between the four tests, 91.6 per cent between the Kolmer-Wassermann, the Kahn and Meinicke tests, 86.8 per cent between the Ruediger-Wassermann, the Kahn and Meinicke tests. This lower percentage was due to the greater sensitivity of the Ruediger-Wassermann test. Schmidt and Ziekman<sup>4</sup> reported the results of 500 consecutive specimens examined by the three tests with 96.2 per cent agreement. The Wassermann and Kahn reactions agreed in 97 per cent of cases, the Wassermann and Meinicke in 97.6 per cent, the Kahn and Meinicke in 97.8 per cent. They concluded that "the Kahn test in this laboratory has not been so sensitive as either the Wassermann or Meinicke turbidity reactions, and in one case a false positivity was obtained. The clinical evidence in this study indicated a higher degree of specificity for the Meinicke turbidity reaction than for the Kahn test."

There are a number of reports comparing the results of the Wassermann and Meinicke tests. Ledermann<sup>5</sup> in an examination of 504 serums found 93

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per cent agreement Rubenstein and Suarez<sup>7</sup> reported 90 per cent agreement in 490 serums, they concluded that the Meinicke test was specific in cases with strong reactions but a little less sensitive than the Wassermann, and that doubtful reactions were not always specific. Saunders<sup>7</sup> reported 94.5 per cent agreement in 1000 blood serums examined. Brugh, Landon and Laid<sup>8</sup> examined 500 specimens finding about 95 per cent agreement between the tests. Blumberg<sup>9</sup> reported 98 per cent agreement in 1500 specimens. Mefford<sup>10</sup> reported that in an experience of over 3000 tests, he found the Meinicke reaction more reliable because it was more sensitive than the Wassermann test. A positive reaction occurred earlier and persisted longer in treated cases. The Meinicke test gave positive reactions in late or recurrent syphilis when the Wassermann test was negative. Cross<sup>11</sup> reported 91.6 per cent agreement in 1500 serums. Rucker and Whitby<sup>1</sup> reported 91 per cent agreement in 254 serums using an antigen prepared by themselves and 90 per cent agreement in 160 serums using Meinicke's own reagent. They considered the Meinicke test easier to perform and to read than the Kahn test. In their opinion the value of the Meinicke test was "mainly for orientation the progress of treatment being controlled by the Wassermann test with or without Meinicke's reactions as confirmatory evidence." Borowsky<sup>12</sup> found 98 per cent agreement between the two tests in an examination of 548 serums.

Our series consisted of 1000 serums the majority of which were chosen because they gave positive reactions with either or both the Wassermann or Kahn tests, which are carried out routinely in our laboratory. The serums were clear, neither distinctly hemolyzed nor chylous they were inactivated for one half hour at 55 to 56° C. after being withdrawn from the clots. If used twenty-four hours, or more later they were reheated at 55 to 56° C. for ten minutes. Meinicke<sup>1</sup> advised using unheated serum while Mefford<sup>10</sup> found that inactivation of the serum made little difference in the results. The Wassermann test was regarded as our standard and Meinicke or Kahn tests not agreeing with it, were repeated.

The technique used in the Wassermann test was similar to that used in the New York State Laboratory. The total volume of the test was 0.5 cc, i.e. one tenth that of the original Wassermann test. All reagents were diluted in accordance with preliminary titrations so that a quantity of 0.1 cc might be pipetted in the test. The complement was titrated daily with 5 per cent sheep cells sensitized with two units of amboceptor and two units of complement were used in the test. Each specimen was tested with two antigens an alcoholic extract of beef heart, reinforced with 0.4 per cent cholesterol, in addition to this antigen, for about 200 of the 1000 tests a crude alcoholic extract of beef heart was used, for the rest we have used an acetone insoluble extract of beef heart prepared according to Bordet's method. Antigens and amboceptor were supplied by the State Laboratory at Albany, New York. The tests with both antigens were kept for four hours at 3 to 6° C. for the period of fixation. Five per cent sheep cells, sensitized with two units of amboceptor were then added and the tests placed in the water bath at 37° C. for fifteen minutes (until controls with serum alone and antigen alone showed complete hemolysis). Readings were then made using a color standard all tubes showing

2+ or more being centrifugalized, and the percentage of inhibition of hemolysis read from the supernatant fluid. About 150 tests were done each day.

The Kahn tests were performed with antigen prepared in our laboratory, Dr. Kahn very kindly checking our titration. The method used differed from the regular Kahn technique<sup>14</sup> only in the use of a two-tube instead of a three-tube test, omitting the tube containing 0.05 cc of antigen. This was done in order to secure uniformity. Many specimens did not provide sufficient serum for three tubes. In addition, from 5 to 10 per cent of the Kahn tests were one-tube tests, using 0.01 cc antigen mixture and 0.1 cc of serum. The result with these proportions of serum and antigen approximated the results of the two-tube test. All tests were read by two workers.

The Meinicke technique was that outlined by Mefford,<sup>15</sup> and the antigen was obtained from him. In view of the statements of Meinicke and others<sup>12</sup> that the efficacy of the antigen is dependent upon the properties of certain preparations of balsam of Tolu, we did not attempt to prepare the antigen in this laboratory. One cc of antigen was placed in one 15 cc centrifuge tube, and 10 cc of 3 per cent salt solution in another, the two tubes were put in a water-bath at 40° C to 45° C for not less than ten nor more than twelve minutes. During this period, 0.2 cc of each serum to be tested was pipetted into a tube. The formalin control was omitted. At the specified time, the antigen and saline were mixed by pouring the saline into the antigen, and back and forth twice. One cc of antigen mixture was added to each tube of patient's serum, while still warm. The tubes were placed in the dark at room temperature for one hour and then read. The positive reactions showed varying degrees of opacity. We did not run controls in this series of tests, but examined the serums themselves for cloudiness, in case of doubtful readings. We read the tests, sitting at about four feet from the window, holding the rack of tubes against the light. To one accustomed to reading Kahn tests, the Meinicke readings were comparatively easy. All tests were read by two workers. The tests were then left overnight at room temperature, and were read again. After this period negatives appeared as unchanged, while strong positives (4+) had a clear fluid and heavy sediment, and 3+ and 2+, were progressively less clear.

#### PRESENTATION OF RESULTS

For our tables, 2+, 3+, and 4+ readings have been grouped as positive, and ±, and + as doubtful.

Table I shows the results of the three tests on 1000 specimens examined. There appears to be very little difference in the results obtained by the three tests. The Kahn test shows a slightly smaller number of doubtful reactions, as read by us.

TABLE I  
RESULTS OF TESTS ON ALL SPECIMENS

	WASSERMANN	MEINICKE	KAHN
Positive	564	560	566
Doubtful	52	59	41
Negative	384	381	393
	1000	1000	1000

Table II shows the percentages of agreement between the three tests, and between each pair of tests. The percentage of agreement between the three tests is 81.5 per cent, which is lower than that apparently indicated by Table I. This is due to the fact that several specimens gave positive reactions with two of the tests while failing to react with the third. Agreement between the Wassermann and Kahn tests and between the Meinicke and Kahn tests appears to be about the same, while that between the Wassermann and Meinicke tests is somewhat lower.

TABLE II  
PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF TESTS

Agreement	WR AND K AND M	WR AND K	WR AND M	K AND M
Positive or doubtful	210	56	46	53
Negative	305	336	312	328
Total Agreements	815 81.5%	592 89.0%	558 83.8%	881 88.1%
Disagreement				
WR pos or doubtful K neg		59		
WR " " M			69	
K " " WR		49		
K " " M				66
M " " WR			73	
M " " K				53
Total Disagreements	185 18.7%	104 10.8%	142 14.2%	119 11.9%
	1000 100.0%	1000 100.0%	1000 100.0%	1000 100.0%

TABLE III  
CORRELATION OF RESULTS WITH HISTORIES

Routine Cases						
	WR.		M		K	
Positive or doubtful	192	38 3%	206	41 1%	193	38 5%
Negative	309	61 7%	295	58 9%	308	61 5%
	<hr/> 501	<hr/> 100 0%	<hr/> 501	<hr/> 100 0%	<hr/> 501	<hr/> 100 0%
Untreated Cases and Cases With Suggestive Histories						
	WR		M		K	
Positive or doubtful	74	69 8%	78	73 6%	70	71 7%
Negative	32	30 2%	28	26 4%	30	28 3%
	<hr/> 106	<hr/> 100 0%	<hr/> 106	<hr/> 100 0%	<hr/> 106	<hr/> 100 0%
Treated Cases						
	WR.		M		K	
Positive or doubtful	350	89 1%	335	85 2%	338	86 1%
Negative	40	10 9%	58	14 8%	55	13 9%
	<hr/> 393	<hr/> 100 0%	<hr/> 393	<hr/> 100 0%	<hr/> 393	<hr/> 100 0%

We divided the thousand specimens according to histories into (1) routines, (2) untreated cases and those with suggestive histories, and (3) treated cases. Routines included those with no history, or a history not suggestive of syphilis. We purposely selected the greater number of our specimens from among those giving positive readings with the Wassermann or Kahn test, as stated previously. Table III shows the results of the three tests on the specimens so grouped. In routine cases there appears to be almost complete agreement between the Wassermann and Kahn tests, which show a smaller number of positives, than



does the Meinicke test, in untreated cases or those with suggestive histories, the results with the three tests agree very closely, while in treated cases, the Wassermann test is more sensitive than the Meinicke and Kahn tests, which agree closely. Briefly, the Wassermann test demonstrates its equality with the other two tests, with the three classes of specimens, and a slight superiority to both, in treated cases.

#### SUMMARY

Our percentage of agreement between the three tests, 81.5 per cent was lower than the figures presented by Ruediger, 87 per cent and 86.8 per cent, and by Schmidt and Zickmann, 96.2 per cent. This may be largely accounted for by the fact that our series contained a much smaller number of specimens which were negative by all three tests.

Our percentage of agreement between the Wassermann and Meinicke tests, 85.8 per cent, was also lower than that reported by others. The reason given above may account for the discrepancy. The cases which disagreed were about equally divided between the two tests.

The Meinicke test showed a few more positive reactions with routine specimens than either the Wassermann or Kahn tests. We have not yet determined, from clinical or other sources, whether or not these were "false positive" results.

In cases with histories suggestive of syphilis and untreated cases of syphilis, the results of the three tests agreed closely.

In treated cases of syphilis the Meinicke test was as sensitive as the Kahn test while both were somewhat less sensitive than the Wassermann test.

#### CONCLUSIONS

The results of the Meinicke's turbidity reaction in the serodiagnosis of syphilis compare favorably with those obtained with the Wassermann technique recommended by the New York State Laboratory. The test is less sensitive than the complement-fixation reaction. In our opinion, it should not be used to supplant the Wassermann reaction at present. Its chief advantages, as compared with a complement-fixation test, are the facility with which it may be set up, and the simplicity and inexpensiveness of the reagents and apparatus required.

The results obtained with Meinicke and Kahn tests are closely parallel.

#### REFERENCES

- 1 Meinicke, E. Flocculation Tests in the Serology of Syphilis, *J LAB & CLIN MED* 12 891, 1926-27.
- 2 Ruediger, E. H. Comparison of the Kahn Precipitation Test, the Meinicke Precipitation Test, and the Ruediger Wassermann Test, *Am J Syph* 11 140, 1927.
- 3 Ruediger, E. H. Comparison of the Kahn Precipitation Test, the Meinicke Precipitation Test, the Kolmer Wassermann Test, and the Ruediger Wassermann Test, *Am J Syph* 11 450, 1927.
- 4 Schmidt, F. R., and Zickmann, R. C. An Analysis of the Wassermann, Kahn, and Meinicke Tests, *Am J Syph* 11 614, 1927.
- 5 Ledermann, R. Vergleichende Untersuchungen über die Reaktionen von Wassermann, Sachs Georgi, Dold und Meinicke, *Arch f Dermat u Syph* 151 236, 1926.
- 6 Rubenstein, M., and Suarez, L. Sérodiagnostic de la Syphilis. Procédé de Meinicke, *Compt rend Soc de Biol* 94 426, 1926.
- 7 Saunders, A. P. The New Modification of the Meinicke Test, *J LAB & CLIN MED* 11 445, 1925-26.

- 8 Brugh, B F, Landon, R H, and Laird E The Meinicke Reaction for Syphilis West Virginia M J 23 No 3 1927
- 9 Blumberg A The Lipoid Fixation Test of Meinicke U S Vet Bur M Bull 3 591 1927
- 10 Mefford W T Technique and Reliability of Meinicke's Turbidity Reaction in Syphilis Clin Med and Surg 34 608 1927
- 11 Gross H Ueber die praktische Brauchbarkeit einiger Trübungs und Flockungs Reaktionen Wien klin Wchnschr 40 1293 1927
- 12 Rucker E A and Whitby, L E H Sero Diagnosis of Syphilis by Meinicke's Macro reaction and Microreaction Brit J Exper Path 9 30 1928
- 13 Borowskaja D Die Meinicke Trübungsreaktion mit gefärbtem Antigen Centralbl f Bakteriell 1st Abt Orig 105 44 1928
- 14 Kahn R L Serum Diagnosis of Syphilis by Precipitation 1926
- 15 Mefford W T Meinicke Test for Syphilis by Precipitation—the Technique for Mixing Materials in the Test Illinois M J 49 210 1926

## A RAPID STAIN FOR THE DIRECT MICROSCOPIC EXAMINATION OF MILK\*

BY NORRIS M ERD B S (HYGIENE) BALTIMORE MD

THE direct method for the enumeration of the bacteria in milk, commonly known as the Breed count has been greatly facilitated in the use by us of a special stain which eliminates many of the time consuming features of the older method, and simultaneously improves the accuracy of the examinations

In the original Breed method 0.01 cc of milk is deposited upon a glass slide, spread over an area of one square centimeter dried, and then stained by a process requiring separate operations of defatting fixation and decolorization

Attempts have been made by others to shorten this technique Newman has recently published three formulas attempting to combine all the procedures outlined in one operation We have not found these stains absolutely satisfactory because considerable difficulty was experienced in obtaining completely defatted milk films within a reasonable time

The following simple technique is submitted therefore for the approval of technical workers in the active milk laboratory Its simplicity is also an advantage in the academic laboratory The milk film is prepared as outlined above The preparations are dried in a warm place upon a level surface and protected from dust In order to prevent noticeable growth the drying should be accomplished within five to ten minutes but excessive heat must be avoided or the dry films may crack or peel from the slides during later handling When dry, the slides are placed in a staining solution having the following composition

### FORMULA

Ether (sulphuric)	50 cc
Methyl Alcohol (absolute)	50 cc
Methylene blue (preferably certified)	0.5 gm

Add the dye to the mixed alcohol and ether When dissolved filter through paper The stain is then ready for use Keep in a tightly stoppered bottle

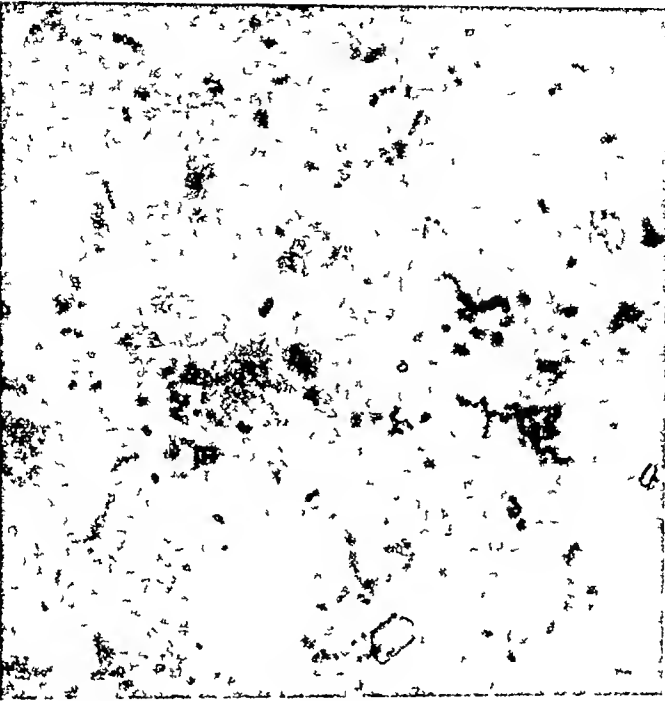


Fig 1

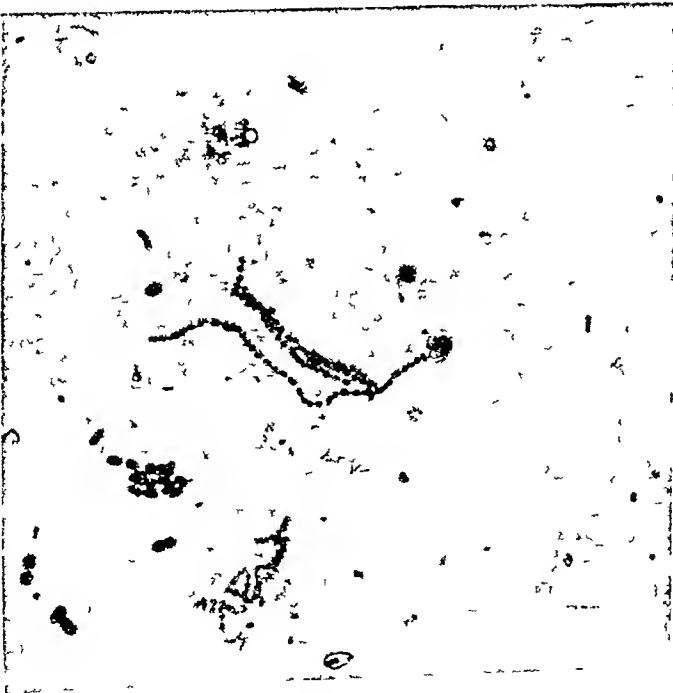


Fig 2

The slides are placed in this staining solution for one minute. They are then rinsed gently but thoroughly in water and dried in air. When completely dry, they may be examined with the standardized microscope as described in Standard Methods of Milk Analysis of the American Public Health Association.

The preparations are defatted, fixed and stained in one operation. There is sharp differentiation between the bacteria and the background. Leucocytes are well stained. The figures illustrate typical microscopic fields stained by this method. Fig 1 is taken from a microphotograph of a pooled specimen of milk having a high bacterial content. Fig 2 is from a routine specimen of milk showing a long chain of streptococci.

#### SUMMARY

1. A one solution stain capable of defatting, fixing and staining milk films in one minute has been described.

2. The direct microscopic count of bacteria in milk is greatly facilitated by the use of this stain.

#### REFERENCES

1. Standard Methods of Milk Analysis. American Public Health Association, Ed 5 1921.
2. Newman R. W. One solution technique for Direct Microscopic Counting of Bacteria in Milk. *Proc Soc Exper Med & Biol* 24: 32-33, 1927.

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## THE WILHITE STAIN FOR NEGRI BODIES\*

By S. W. BOHLS, M.D., AUSTIN, TEXAS

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THE following formula for staining negri bodies in rabie brains was developed by Dr J. T. Willhite, Director of the Texas State Pasteur Institute of Austin. Since his death in 1927 we have continued to use it with great success.

The formula is as follows:

Methylene Blue	0.9 gm
Rosaniline Violet	1.5 gm
Glycerine	125.0 cc
Methyl Alcohol	125.0 cc

Mix, shake and let ripen. Make impressions from the Ammon's horn on a glass slide, then take ten drops of the stain with 20 cc of 1:50,000 KOH and stain two minutes with heat. Dry and examine under the microscope. The Negri bodies will take the acid stain. Always make fresh stain every morning.

PASTEUR INSTITUTE.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE, M D , ABSTRACT EDITOR

## LABORATORY TECHNIC

**HYDROGEN ION** A Universal Indicator for Hydrogen Ion Concentration, Bogen, E J  
A M A 89 199, 1927

The following mixture has also been developed for this purpose, as it has a range from  $P_H$  1.0 to  $P_H$  10.0, with a continuous change of color with change in hydrogen ion concentration phenolphthalein, 100 mg, methyl red, 200 mg, dimethylaminoazobenzene, 300 mg, bromthymol blue, 400 mg, thymol blue, 500 mg Dissolve in 500 cc of absolute alcohol Add tenth normal sodium hydroxide solution until the red disappears and the solution becomes yellow ( $P_H$  6.0)

For determining the hydrogen ion concentration of any solution, add one drop of this indicator to 1 cc of the specimen The colors produced resemble those of the spectrum, thus

Red	indicates about $P_H$ 2.0	(very strongly acid)
Orange	indicates about $P_H$ 4.0	(strongly acid)
Yellow	indicates about $P_H$ 6.0	(weakly acid)
Green	indicates about $P_H$ 8.0	(weakly alkaline)
Blue	indicates about $P_H$ 10.0	(strongly alkaline)

For closer approximation, a series of standard solutions for comparison can be made with known buffer mixtures as described by Clark and Lubs

**LEPTOSPIROSIS** The Early Diagnosis of Leptospirosis, Schuffner, W Munchen med  
Wchnschr 73 1977, 1926

Two to 3 cc of blood are withdrawn by cardiac puncture from an inoculated guinea pig into a syringe containing citrate solution The mixture is centrifuged five to six minutes at 1500 rpm Pipette off the plasma and centrifuge it for ten minutes at 1500 rpm The plasma is again transferred to a new tube and centrifuged thirty minutes at 3000 rpm The plasma is decanted and a drop of sediment examined by dark field

**TUBERCULOSIS** Simultaneous Demonstration of Tubercle Bacilli and Elastic Fibres,  
Jessen, F Beitr z Klin d Tuberk 65 4, 1926

When there is plenty of sputum available about 4 cc of sputum is mixed with 10 cc of sodium hydroxide With smaller amounts of sputum only 5 cc of normal sodium hydroxide is used This mixture of sputum and sodium hydroxide is heated over a water bath for twenty minutes at a temperature not higher than 50°, stirring it lightly with a glass rod It is then centrifuged A smear of the sediment is allowed to dry, preferably in the incubator, and fixed in a flame It is stained with carbol fuchsin, heated as usual, and washed, decolorized with 3 per cent alcoholic solution of hydrochloric acid, as usual, and washed, then stained one to two minutes with hematoxylin and cold saturated solution of lithium carbonate, each 10, absolute alcohol and distilled water each 20.0 and then washed They are then decolorized with official iron chloride solution for a few seconds and washed With this method the tubercle bacilli are stained a beautiful red and greater numbers of them show than with the ordinary method The author has often demonstrated bacilli by this method in specimens in which they could not be demonstrated by the ordinary methods The elastic fibers are bluish gray

**TUBERCULOSIS** Bacteriologic Diagnosis in Infants by Examination of the Stomach Contents Armand Delille P F Am J Dis Child 34 No 4, 547, 1927

About 80 cc of tepid water is introduced into the stomach and withdrawn by siphoning. This is accomplished by means of a stomach tube which has been lubricated with albolene or olive oil, and which has a tube or funnel attachment. In some cases in which real cavities are found in the lung purulent sputum is obtained while in other cases in which bacteriologic examination is particularly important only a few flecks of mucus are seen.

If characteristic sputum is obtained direct microscopic examination by the Ziehl-Neelsen method is all that is necessary but in most cases homogenization is required. The liquid is centrifugalized in four tubes and the precipitates united in a porcelain dish to which is added 30 cc of water and 10 drops of normal sodium hydroxide. The whole is heated for ten minutes, 50 cc of water being added slowly. If the specific gravity is over 1.004 a little alcohol is added. The material is again placed in four tubes and centrifugalized for forty-five minutes and the precipitate is stained by the Ziehl-Neelsen method. The stomach contents can be kept two or three days before homogenization without destroying the tubercle bacilli. Microscopic examination must be carefully made because in some cases it is rather difficult to find the bacilli.

The results obtained by this method have been interesting. In sixty-two cases in which only the direct examination without homogenization was used, tubercle bacilli were found in only 10 per cent while in 110 cases in which the method here detailed was employed 31 per cent were positive.

**TUMORS** The Relation Between the Histologic Picture and Prognosis of Tumors Plant A. Arab Path & Lab Med 3 240 1927

The present possibilities of histologic prognosis are smaller than would appear from the examples given. Conclusions have been drawn from follow-up investigations but in only a small number of these cases has it been possible to make a histologic prognosis from an excised tumor fragment. Histologic prognosis can be safe only when the tumor is homogeneous and when it runs true to course that means when it does not change its structural and biologic character during the progress of the disease. Carcinoma of the cervix, for instance, fulfils neither of these conditions in a sufficient degree.

General histologic prognosis is feasible in rare instances only. More promising are the endeavors to establish histologic prognosis for special tumors in special locations. The method of clinical statistics is subject to great errors and only very large figures from homogeneous material which are obtained by the same method throughout can be made the basis for positive conclusions. The many contradictory statements quoted above show that only too well. After extensive studies in such special histologic prognosis it may be possible in the future to determine which rules for general prognosis will remain perhaps it will be only those governing the bad prognosis to be made from the highly irregular pleomorphic structures.

The interpretation of the most used histologic features is so far uncertain. This is true of inflammation in the stroma and in the epithelium itself, maturity of cells and anaplasia and also of the mass relation between epithelium and mesenchyme.

Knowledge of cell division in tumors requires much more study with special reference to the occurrence of anutosis, of intermediate forms of cell divisions and of the different phases of true mitosis. The distribution of the cell divisions may be important also. In order to discuss the term 'malignancy' it is necessary to consider separate local extension, metastasis, recurrence, cachexia, local regression during irradiation and occurrence of metastasis after irradiation. Constitutional factors such as age, race and sex must be studied in their interrelations with microscopic pictures.

Histologic prognosis can be a great help to the physician if he is always aware of its limitations. There may be disappointment sooner or later when prognoses are made from microscopic slide examination according to standardized rules. Only the most careful study of individual cases can avail us in the presence of the manifold histologic and clinical phases of malignant tumors.

**AMYLOID DISEASE** The Clinical Value of Intravenous Injection of Congo Red in the Diagnosis of Amyloid Disease, Bookman, A, and Rosenthal, J Am J M Sc 173 No 3, 396, 1927

In a study of 18 cases the authors have found that disappearance of 60 per cent or more of congo red from the blood in one hour is found only in the presence of amyloid disease. A lower rate of disappearance does not preclude the presence of amyloidosis.

One and two tenths grams of powdered Congo red (Grubler) is mixed with 100 cc of water, heated to boiling, filtered through a Berkefeld filter and divided into hard glass ampules each containing about 15 cc. These are then sealed. The solution must contain no undissolved particles. Ten to 15 cc are injected, according to the size of the patient, into a vein at the elbow. The needle is left in place and after four minutes 10 cc of the blood are withdrawn. At the end of one hour a second specimen of 10 cc is taken.

Serum (which must be free from hemoglobin) is obtained by bleeding through a dry needle into a paraffin tube which is quickly transported, packed in ice, to the laboratory. The blood is immediately transferred to an unparaffined centrifuge tube and centrifuged at 1500 to 2000 revolutions per minute for at least fifteen minutes. The fibrin is then detached from the walls with a needle, being careful not to approach within a centimeter of the red cell zone. The clot is allowed to retract and the tube again centrifuged for five minutes. The serum is then pipetted off and the color content of the one hour specimen determined with any colorimeter, the four minute specimen being used as a standard. The Kuttner-Leitz Universal microcolorimeter is most useful for this purpose. The addition of dilute hydrochloric acid to the serum changes the color to blue. At times this was found of advantage as a check on the matching of the red color.

**CEREBROSPINAL FLUID** Night-Blue Reaction for the Determination of Albumin Increase in Spinal Fluid in Meningitis, Rosenfeld, H Klin Wchnschr 6 118, 1927

Two solutions are required for the reaction.

1. A one per mille aqueous solution of night blue. This solution can be preserved for a long time in a buffer mixture (the hydrogen ion concentration being 4.6) consisting of 110 cc N/10 acetic acid, 90 cc of N/10 sodium acetate, 200 cc of distilled water. The constancy of the reaction must be controlled from time to time.

The reaction is made as follows. Solution 1 is 10 times diluted with distilled chlorine free water and equal parts of this dilution and of the buffer mixture mixed with each other. Two tenths cc of the cerebrospinal fluid to be examined are put in an acid and alkali free dry test tube, filling up to 1 cc with distilled water. Two cc of the night blue buffer mixture are added. As a control 1 cc of distilled water and 2 cc of night blue buffer mixture are put in another test tube. Both tubes are slightly shaken and compared with each other.

In case of meningitic cerebrospinal fluid, rich in albumin, a diffuse turbidity appears immediately. In normal cerebrospinal fluid or one which is rich in globulins (syphilis, tabes, paralysis), however, and in the control tube the mixture remains transparent, blue and clear. Cerebrospinal fluid containing blood also renders a positive reaction because of its marked serum albumin content.

From a study of 380 cases the author believes this test of distinct value.

**TISSUE STAIN** Improved Method of Preparation of Weigert's Elastin Stain, Haynes, F J Roy Mic Soc 46 29, 1926

To 1 per cent aqueous basic magenta solution add 2 per cent of phenol. Bring to boiling and add an excess of 50 per cent solution of ferric chloride. Boil ten minutes, collect, wash, and dry the precipitate. For use 0.75 gm is added to 100 cc of 96 per cent alcohol containing a few drops of concentrated HCl, boil for fifteen minutes, cool, filter, and add 2 per cent of concentrated HCl. The powder keeps indefinitely and the staining solution two months.

**STAINS** The Germicidal Effect of Staining Solutions Eckfeldt G. A. and Loser S. A. *Stain Technology* 2 No 4, 100, 1927

Gentian violet, crystal violet and carbol fuchsin applied to cover slip preparations for one minute will destroy the majority of nonspore forming bacteria and yeasts, though they cannot be relied upon to do this consistently and in all cases

The Gram staining procedure is more effective and nonspore formers were never found to survive this process

Methylene blue stains exert very little if any germicidal power and most organisms survived them readily India ink was totally ineffective

**NEPHRITIS** Diazo Test in Nephritis Blatner H. and Fitz R. J. *A. M. A.* 88 985 1927

To 1 cc of blood plasma or serum is added 2 cc of 96 per cent alcohol. The proteins are allowed to precipitate and the mixture is filtered or centrifugalized. The clear filtrate, or supernatant fluid from the centrifugalized specimen is then collected. To 1 cc of this clear fluid is added 0.5 cc of alcohol and 0.25 cc of freshly prepared diazo reagent. The mixture is boiled for thirty seconds and then a few drops of a 10 per cent solution of sodium hydroxide are added.

The characteristic reaction is the very rapid development of a pink color, which may disappear rapidly. The intensity of the color and the rate of its disappearance depend upon the concentration of the substance causing the reaction in the blood. The mixture must be watched very carefully during the addition of the alkali, since the pink may appear and disappear within a few seconds. The diazo reagent can be prepared according to the directions of McNee. It consists of a mixture of two solutions each of which keeps well but which must always be freshly made immediately prior to a test. The two solutions are prepared in the following fashion:

**Solution A** sulphamic acid, 1 cc concentrated hydrochloric acid, 15 cc distilled water, 1000 cc

**Solution B** sodium nitrite 0.5 gm, distilled water, 100 cc

The reagent as used for the test consists of 25 cc of Solution A, to which is added 0.75 cc of Solution B.

The authors believe a positive reaction to be of ominous significance.

**BLOOD CHEMISTRY** A Note on the Preservation of Samples of Blood for Sugar Estimations Splatt, B. M. *J. Australia* 2 178, 1926

Confirming the suitability of the method of preservation described by Sanders (5 mg of 5 l mixture of sodium fluoride and thymal per 0.2 cc of blood)

**ASTHMA** A Comparative Study of the Scratch and Intradermal Methods of Skin Testing in Children Peshkin, A. M. and Fineman, A. H. *J. Dis. Child* 34 No 5, 815 1927

Ninety one children with asthma and nine with eczema, urticaria or angioneurotic edema, ranging in age from eight months to fourteen years were tested by the scratch and intradermal methods with thirty three corresponding proteins. Dry powdered extracts (Wodehouse) were employed for the scratch and routine fluid extracts (Coca) for the intradermal tests. The fluid extracts were also applied with the scratch method when the intradermal test gave a reaction above the control. All of these children were under observation for at least one year.

In interpreting the reactions obtained with the scratch method, all reactions above the control were considered positive. With the intradermal reaction although all reactions above the control were recorded only reactions of two plus or more were considered definitely



positive This interpretation is in accordance with accepted standards for this method However, it appears that one plus reactions should not be entirely ignored in spite of the fact that they are most frequently nonspecific

Thirty eight children of the entire series reacted negatively with the scratch method With the intradermal method, fifteen children gave negative reactions, and in twenty three the reactions obtained were plus minus and one plus only

Sixty two children reacted positively to the scratch method With the intradermal method, twenty six patients gave reactions not larger than one plus and in thirty six two and three plus

No patients were encountered who gave definitely positive reactions with the intradermal method and negative reactions with the scratch method

The total number of negative reactions obtained with the scratch method were 3,032 The corresponding reactions with the intradermal method were definitely positive ( $++$  and  $+++$ ), in 0.53 per cent, one plus, in 4.15 per cent, and negative ( $-$  and  $\pm$ ), in 95.32 per cent

The total number of positive reactions ( $\pm$  to  $++++$ ) obtained with the scratch method was 233 The corresponding reactions with the intradermal method were definitely positive in 19.31 per cent, one plus in 27.47 per cent and negative in 53.22 per cent

Pseudoreactions or nonspecific reactions were common with the intradermal method Practically all the plus minus and a majority of the one plus reactions were of this character The nonspecific reactions occurred most commonly with the following substances in their order of frequency, house dust, chicken meat, rice, wheat, chicken epithelium, tobacco, corn, pyrethrum (insecticide), goose and goat epithelium and lamb Pseudoreactions with the scratch method were relatively infrequent

Four children gave a history of positive pollen reactions and negative ragweed reactions with both methods of testing (the fluid extract for the intradermal test was employed up to a concentration of 0.25 mg of nitrogen per cubic centimeter), they gave positive reactions by the conjunctival test to the dry powder The latter method testing has been shown by Peshkin to be a rapid, safe and reliable method of demonstrating sensitivity to pollen in patients refractory to skin tests

General reactions occurred in 2 per cent of the patients tested with the intradermal method and none with the scratch method

The scratch test is superior to the intradermal test for the following substances rabbit hair, horse dander, ragweed, cottonseed, duck and goose feathers, cat and dog hair, mustard, and egg white

The intradermal test is superior to the scratch test for the following substances house dust, chicken epithelium, chicken meat, wheat and corn In the presence of negative scratch reactions to horse serum and goat hair, the intradermal method, in a small percentage of the cases will reveal definitely positive reactions

From the standpoint of safety and the determination of etiologic sensitizations, the scratch method would appear to be superior as a routine procedure for testing The value of the intradermal method cannot be ignored One method should not be employed to the exclusion of the other

## REVIEWS

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Books for Review should be sent to Dr Warren T Vaughan Medical Arts Building  
Richmond Va

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### *Daily Consultations Les Consultations Journalieres\**

A SERIES of small handy reference volumes on subjects of interest to the general man and to those interested in the special lines to which each volume is devoted. Their special interest to the American students lies in their exposition of the French viewpoint on the subjects discussed. Radiotherapy including x ray and radium treatment and ultra violet ray therapy is covered by W Vignal electro radiologist of the hospitals of Paris. This is a volume of 425 pages in which therapy is discussed in the deepest detail, from its beginnings. Little attention is given to diagnostic roentgenology.

The volume on gynecology is contributed by E Douay Chief of Gynecology at the Broca Hospital. This volume is decidedly more monographic than the former as it deals with four selected phases of gynecology. These are menorrhagia and metrorrhagia considered as one leucorrhea dysmenorrhea and sterility. The first three as the author points out are the three great symptoms that dominate all gynecology. The discussion of all four subjects is comprehensive and many valuable therapeutic points are to be found in the volume.

A rather large volume on diseases of the esophagus stomach and duodenum by Maurice Delort, and on diseases of the oral cavity including the teeth by F Nidergang both of Paris, forms an excellent reference manual. The illustrations particularly the radiograms are well reproduced.

An index of therapy entitled *Les Formules Usuelles* by Segard and Laemmer takes up the various commoner diseases in symptomatic and pathologic classification using an alphabetic order. Under each subject the outline for therapy including many special prescriptions is presented in tabular form.

No one man will have need for all of the volumes but the internist or general practitioner who has an acquaintance with French will find much of value in the last three volumes mentioned.

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### *Les Indications Cliniques de L'Electro Radiotherapie †*

THIS is a new idea. The author includes electrotherapy diathermy radiothermotherapy phototherapy actinotherapy x ray and radium treatment and in the discussion of each clinical condition designates which of these methods of treatment or what combination should be utilized. Technique is included. Statistical results are not included.

Daily Consultations *Les Consultations Journalieres* G Doin & Co Paris 1927

†*Les Indications Cliniques de L'Electro-Radiotherapie* by Etienne Piot Paris Librairie Octave Doin Gaston Doin & Cie Editeurs 8 Place de L'Odeon 8 1927

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NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

### *The Clinical Interpretation of Blood Chemistry\**

IT IS not essential that the general practitioner or the clinician be adept in the manipulative technicalities of blood chemistry studies. He should, however, be thoroughly familiar with the significance and clinical interpretation of the findings. Unfortunately the latest information on the clinical significance is not always readily available since the reports often appear in highly specialized journals and are widely scattered through the literature. The author has collected the necessary information in this small volume. It is not a laboratory manual and gives little detail of laboratory procedure but starts the study of each blood chemical determination at the time when, the laboratory work being completed, the final report has been made.

In addition the author includes a large variety of food tables which are thus available for handy reference, and a general outline for the management of those diseases in which the blood chemistry shows alterations.

*Self-Care for the Diabetic\**

### *Self-Care for the Diabetic†*

A MANUAL for the use of diabetic patients which follows the same general line as the several similar volumes which have been developed in this country and should be of interest to us in the United States as illustrating the British attitude toward the treatment of this highly important subject. The reviewer feels that the volume has one shortcoming in that it does not provide ample facility for the patient to calculate his own diet and to provide himself with a wide variety of foods while following his dietary prescription.

### *The Carrier Problem‡*

IN THE realm of the infectious diseases the carrier problem is as true and as pressing a problem as any that exists. And yet surprisingly few have made any extended special studies in this field. The last monograph on this subject appearing in England prior to the one under review appeared as long as sixteen years ago. The present author makes no claim to inclusiveness in his discussion of the subject but presents the outstanding facts of the carrier stage as we know it today. The work covers the enteric infections, diphtheria, meningococcus infection, pneumococcus and streptococcus infections, poliomyelitis, bacillary dysentery, cholera, protozoan infections, and a small group of miscellaneous diseases. Under each he describes the probable development of the carrier state and discusses those methods which we have at present at our disposal for the prevention and relief of this condition. His conclusion is that at present we know distressingly little concerning the mode of development of the carrier state and even less of its cure.

And yet in epidemiology the subject is of utmost importance for were we able to recognize and cure the carrier these diseases would stand in good way of being eliminated. Yellow fever and malaria neither of which the author discusses, for he is dealing primarily with human carriers, are examples of the great potentialities in the hands of preventive medicine provided a satisfactory method of eliminating the carrier is found.

The carrier problem is not alone for the epidemiologist. Few states call for as closely coordinated study by a wide variety of interests. The bacteriologist, the immunologist, the protozoologist and the public health official must all cooperate and for greatest success the family physician whose contact with the individual is the most direct should cooperate as thoroughly.

\*The Clinical Interpretation of Blood Chemistry. By Robert A. Kilduffe, A.B., A.M., M.D., Director Laboratories Atlantic City Hospital; Consulting Serologist Betty Bacharach, Home for Children; Serologist Jewish Seaside Home; City Bacteriologist Atlantic City. Formerly Director Laboratories Pittsburgh Hospital, Pittsburgh, Pa. Cloth. Pages 186. Lea & Febiger, Philadelphia, 1927.

†Self-Care for the Diabetic. For the Use of Diabetic Patients. By J. J. Conybeare, M.C., M.D., Oxon., F.R.C.P. (Lond.), Assistant Physician to Guy's Hospital. Pages 70. Cloth. Oxford University Press, American Branch, N. Y., 1926.

‡The Carrier Problem. By K. C. Paul, M.B., B.S., Sometime Research Student in Medicine, University of Madras. With foreword by David Nabarro, M.D., F.R.C.P. (Lond.), Director of the Research and Pathological Laboratories, The Hospital for Sick Children. Pages 102. Cloth. Oxford University Press, American Branch, N. Y., 1926.

While vast sums are being spent yearly on endowments for medical research the reviewer would suggest the establishment of an institute primarily for the study of the carrier state, an institute which might with benefit adopt certain features of the Framingham tuberculosis demonstration and of the James Mackenzie Institute. In other words it would be located in a relatively isolated, unchanging community, be provided with the best of laboratory facilities, and would elicit the hearty interest and cooperation of the practicing physicians in the community. For best results the study of the carrier problem will necessarily require concomitant laboratory investigations and clinical application directed to the individual with contemporary epidemiologic supervision applied to the group or community.

### *The Enlarged Prostate \**

A VOLUME devoted primarily for the urologic surgeon. While the usual method of treatment remains prostatectomy either suprapubic or perineal the author reports a number of instances of successful results by partial removal by means of perurethral operation, usually with diathermy. He also mentions the good results that have been obtained particularly in Germany following x-ray treatment. He remarks that previous x-ray makes subsequent operation no more difficult but that it appears in some cases to delay healing of the abdominal wound.

The volume makes a quite complete work on the prostate gland taking up as it does the anatomy and physiology of the prostate, the pathology of enlargement, symptoms, diagnosis and treatment of hypertrophy.

Prostatectomy is at no time a simple or easy procedure. It carries with it a definite mortality. Walker quotes a large series of records in which he shows that skill decidedly lessens operative mortality. The death rate in clinics devoted entirely to genitourinary surgery runs between one third to one half the death rate in otherwise equally efficient and well recognized general hospitals. At St Thomas' Hospital, London, the mortality was 20.3 per cent, at St Bartholomew's 22 per cent while at St Peter's, a special hospital devoted entirely to the treatment of genitourinary diseases the death rate was 9.28 per cent.

### *The Treatment of Chronic Arthritis and Rheumatism †*

A RATIONAL presentation of our present understanding of the problem of arthritis and allied diseases. The authors have no pet theory nor an unusual method of treatment. They present various therapeutic measures more generally used for what they are worth. The authors do stress the importance of recognizing the various types of joint disease and instituting the treatment appropriate for the particular type. The differentiation of types and sub-varieties is well worth the reading by all who are interested in this subject. A chapter on common mistakes in diagnosis emphasizes the importance of ruling out other types of pathology such as static deformities, newgrowth, diseases of the central nervous system, certain diseases of bones and joints, and referred pain due to visceral disease. Neuritis and Neuralgia\*.

### *Neuritis and Neuralgia ‡*

AN EXHAUSTIVE and informative treatise on disease of the peripheral nerves and neuralgia of central origin such as migraine, which will be of especial value to the neurologist and the neural surgeon.

*The Enlarged Prostate* By Kenneth M. Walker F.R.C.S. M.A. M.B. B.C. Jacksonian Prizeman and Hunterian Professor Royal College of Surgeons 1911 1922 and 1934. Lecturer in Venereal Diseases St Bartholomew's Hospital Surgeon with charge of Genito Urinary Department, Royal Northern Hospital Surgeon to St. Paul's Hospital. Cloth. Illustrated. Pages 193. Oxford University Press. American Branch New York, 1926.

†*The Treatment of Chronic Arthritis and Rheumatism* By H. Warren Crowe D.M. B.Ch. (Oxon.) M.R.C.S. L.R.C.P. Cloth. Illustrated. Pages 187. Oxford University Press. American Branch N.Y. 1927.

‡*Neuritis and Neuralgia* By Wilfred Harris M.D. Cantab. F.R.C.P. Lond. Senior Physician to St Mary's Hospital and to the Hospital for Epilepsy and Paralysis, Maida Vale. Cloth. 418 pages. Illustrated. Oxford University Press. American Branch New York.

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## EDITORIALS

### *The Statistical Study of Diabetes*

KNOWLEDGE, in the last analysis, depends upon the accumulation, digestion, and interpretation of experience and especially is this true in the development of diagnostic and therapeutic acumen. For this reason the careful survey of any large series of observations is of great value to the physician at large, and such a survey of diabetes, embracing two thousand cases, has recently been published by John<sup>1</sup>

Despite the volume of work which has been done the benefit which has accrued to the diabetic in comfort, in the avoidance of complications, and in the prolongation of life, there is still much to be learned and much to be done before any diabetic in any place may be assumed of competent and intelligent handling.

The use of insulin, for example, is now widespread but it may be questioned if a thorough knowledge of the minutia of insulin treatment essential to therapeutic success is equally widespread.

John's report contains much that is of interest to the physician at large and it is, for that reason, herewith summarized

The incidence of diabetes among all diseases seen during the course of this study (87,449 cases from 1921 to 1927) was 2.28 per cent. The incidence as to sex was males 46.75 per cent and females 53.25 per cent. The age incidence was highest in the fourth decade although most of the cases came from the fifth, sixth, and seventh decades.

There has been much speculation as to the importance of various factors in the etiology of diabetes. As John comments, the fact that obesity usually first manifests itself in the decade in which the incidence of diabetes is greatest, the fourth, forces some thought as to the exact relation one bears to the other. Infection, also, has been regarded by some as a predisposing factor of greater or less importance. In the present series however as infection predominates in the first two decades in which the incidence of diabetes is low, its marked importance as an etiologic factor may be doubted in John's opinion.

There was a hereditary history of diabetes in 5.3 per cent and a familial history in 4.5 per cent, figures somewhat lower than those usually encountered in the literature.

The highest blood sugar noted was 900 mg per cent.

The observations as to the correlation between glycosuria and hyperglycemia are of interest to the laboratory worker who must explain at times that the absence of the expected correlation does not necessarily predicate technical error in the laboratory.

In 159 cases in which glycosuria was present the blood sugar was below 180 mg, and in 26 cases with glycosuria the blood sugar was only 120 mg per cent. In one case on the other hand there was no glycosuria with a blood sugar level of 390 mg per cent and others though not so striking which illustrate how relatively impermeable the renal filter is to dextrose.

As John says "The point which one must derive from these data is that the blood sugar level does not by any means disclose the level of the renal threshold.

"The urine collected at the moment when the blood is taken for sugar estimation is not the urine which has been secreted during those particular few minutes, but rather includes that urine plus all the urine which was secreted during the period of time since the last preceding voiding. It is necessary therefore to determine what the blood sugar level was throughout that period. Did it reach a high level above that of the renal threshold, and gradually drop to the level at the time the specimen was taken, or was it at the same level at which it was found throughout the period since the last preceding voiding? The figures per se do not give definite information as to the patient's renal threshold. It is interesting however to observe that in a good many definitely diabetic patients in whom the blood sugar content is low glycosuria is present."

John presents some very pertinent observations upon insulin treatment. He says "In the treatment of persons with diabetes there are four chief

objectives (1) To get the patient as nearly normal as possible, (2) to find out what is required to gain and maintain the normal state, (3) to plan a livable, not a starvation, diet so that the patient may hold his job, and (4) to instruct the patient in the routine he must follow if he is to maintain the best possible status "

Between simple starvation with all the consequences it entails besides lowering the blood sugar, and a livable diet from the start with enough insulin to lower the blood sugar to normal as desired, John favors the latter method as the more economically logical and more conducive to the general well-being of the patient. And he emphasizes that it is not the diet but the insulin which must be juggled to accomplish this end. Inasmuch as, he says, insulin loses its effect relatively when the dosage is increased, one should employ the smallest amount which will accomplish the desired result.

Those who fear that once begun insulin must be used forever will find comfort in the repetition of the fact, emphasized anew by John's series, that this belief is a fallacy.

The observations of John concerning the treatment of coma are of practical interest.

He comments upon the common belief that when a diabetic is unable, for one reason or another, to take food, starts vomiting, and enters the path leading to acidosis, it seems logical to withhold insulin for fear of hypoglycemia, thus increasing the likelihood of coma. The physician, however, must forcibly impress upon the patient and his attendants the fact that the body continues to burn calories just the same, the only difference being that when food is taken the calories come from without whereas they are supplied by the burning of the body tissues in the absence of food. In either case insulin is required to accomplish the combustion so that vomiting is not a sign for the cessation of insulin but an evidence of the need for insulin.

In the treatment of coma John believes that three points should be stressed.

"1 The administration of the sufficient amount of insulin to produce the desired effect of clearing up acidosis and lowering the blood-sugar level, this may take anywhere from 80 to 700 units in twenty-four hours

"2 The use of 10 per cent dextrose intravenously

"3 Hospitalization of the patient "

In the treatment of coma there has been some difference of opinion as to the advisability of administering glucose intravenously with insulin because, as has been said, this simply increases the hyperglycemia. But, as John points out, if the amount of insulin is calculated in accordance with the total amount of glucose in the blood, not only will there be a fall rather than a rise,<sup>2</sup> but in the combustion of the total glucose the acetone bodies will be consumed, and experience has shown that large and frequent doses of insulin will be absorbed by the patient without difficulty.

New data are brought by John illustrating what indeed requires appreciation the variability of individual response to insulin and the necessity

in some cases for what he calls 'the wearing out process' meaning the strict adherence to routine in cases showing a slow uneven progress, but in whom eventually a normal state may be established and, once obtained, maintained by a remarkably small amount of insulin

John calls attention to the brilliant results now possible in the surgery of diabetes by careful pre and postoperative treatment with insulin and glucose intravenously

The paper above thus briefly summarized constitutes a contribution to the subject of diabetes which well repays study in the original

## REFERENCES

- 1 John, H J Diabetes A Statistical Study of Two Thousand Cases Arch Int Med 42 No 2 217, 1928
- 2 John, H J Diabetic Coma Complicated by Acute Retention of Urine J A M A 84 1400 1925

—R A K



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## News Items

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The Registry of Technicians under the auspices of the American Society of Clinical Pathologists is being conducted in the Office of the Society under the direction of a Board of Registry. This Bureau also includes an employment service. Information and application blanks may be obtained by addressing the Registry of Technicians of the American Society of Clinical Pathologists, Metropolitan Building, Denver, Colorado

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AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

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## FURTHER OBSERVATIONS WITH A NEW METHOD FOR CULTIVATING TUBERCLE BACILLI A COMPARISON WITH GUINEA PIG INOCULATION AND PETROFF'S METHOD\*

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By H J CORPER M D PH D AND NAO UYEI PH D, DENVER, COLORADO

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### PART I

UP TO the present time two methods have surpassed culture methods for the detection of tubercle bacilli in the experimental and clinical study of tuberculosis. The microscopic examination for stained bacilli in smears or sections is the most favored probably because of its ease of manipulation but it lacks the delicacy of the more expensive guinea pig inoculation method. In the experimental laboratory the culture method has proved itself adaptable to many uses and a valuable means for isolating bacilli, thus making it unreplaceable by either staining methods or animal inoculation. The satisfaction of watching a growth of bacilli develop on the culture medium is gratifying especially in comparison with any method of animal inoculation. Since, however, culture methods in the past merited neither speed nor delicacy it was hardly to be expected that they would come in general practical use. In earlier publications<sup>1</sup> however a new method was described by us for the isolation of tubercle bacilli from contaminated tuberculous materials which in preliminary tests fulfilled the requirements necessary to suggest the possibility of its use as a substitute for the guinea pig inoculation method. At that time it was reported that of the various reagents used for the primary isolation of tubercle bacilli from contaminated tuberculous materials sulphuric acid and hydrochloric acid were found superior to any other reagents tried, including sodium hydroxide for destroying the undesirable contami-

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Research Department National Jewish Hospital at Denver Colorado

Read before the Seventh Annual Convention of the American Society of Clinical Pathologists Minneapolis Minnesota, June 8 9 and 11 1928

nators present Standard crystal violet or methyl violet of the American dyes was found serviceable in replacing the prewar Grubler's gentian violet, and in the same concentration, for preparing Petroff's gentian violet egg medium Crystal violet potato cylinder medium was found superior to gentian violet egg medium, Dorset's egg medium, Petroff's gentian violet egg medium, glycerol-agar and Long's nonprotein medium, in favoring the growth of the bacilli when present in small numbers The other media were found serviceable in this respect in the order given above The new method for the isolation of tubercle bacilli consisted in using an equal volume of 6 per cent sulphuric acid at 37° C for thirty minutes in the preliminary treatment of the tuberculous material, and crystal violet potato cylinder medium for culturing the bacilli The crystal violet potato medium was prepared by immersing clean potato cylinders in 0.0015 per cent standard crystal violet in a 1 per cent solution of sodium carbonate for one to two hours and autoclaving the potato cylinders for thirty minutes at 15 pounds pressure after placing in a sterile culture tube containing 15 c c of 5 per cent glycerol broth Since the appearance of our earlier reports, we have been engaged in attempts to simplify the medium and to evaluate the new method further in practical tests with sputums, urines, and animal tissues The following report will be concerned with the findings obtained thus far in these studies

#### ATTEMPTS TO IMPROVE AND SIMPLIFY THE CRYSTAL VIOLET POTATO CYLINDER GLYCEROL BROTH MEDIUM

Since the original potato cylinder medium containing the 15 c c of glycerol broth did not appear to us to be as convenient as an entirely solid medium for isolating tubercle bacilli, an attempt was made to prepare a medium in which ground potato was incorporated with agar-agar and in which other ingredients were used in varying proportions for the purpose of learning the most efficient combinations suited to the purpose of supporting the growth of the tubercle bacilli

The first experiments in this series were concerned with finding the most suitable combination of ground potato, glycerol, and broth in agar-agar for enhancing the growth of tubercle bacilli

*a The Optimum Proportion of Ground Potato in Glycerol Agar Mediums for the Growth of Tubercle Bacilli*—A standard medium ("S") consisting of 13.4 per cent of 4 times ordinary strength (4x) broth (containing 12 grams of beef extract, 40 grams of peptone, and 20 grams of sodium chloride diluted to 1000 c c with tap water), 20 per cent pure glycerol, and 1.1 per cent agar-agar were mixed with different proportions of freshly ground potato (ranging from 5 to 60 per cent) and distilled water After mixing well, the medium was neutralized by means of 10 per cent sodium carbonate (c p anhydrous) solution using litmus paper as a test The mixture was then sterilized in an autoclave at 15 pounds pressure for thirty minutes (excessive heating was avoided as this has been found to have a destructive influence upon the growth promoting properties of the potato for tubercle bacilli) In order to test the efficiency of these mixtures for supporting the growth of tubercle bacilli, the tubes of medium were seeded with three different strength sus-

pensions of virulent human tubercle bacilli (Gluckson), containing 1 mg, 0 000,1 mg or 0 000,001 mg of bacilli per cubic centimeter

The results of these cultural studies after four weeks with the 10 mg per cc seeding, six weeks after the 0 000,1 mg per cc seeding, and ten weeks after the 0 000,001 mg seeding are recorded in Table I using as control the potato cylinder medium without crystal violet

TABLE I

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GLYCEROL AGAR NUTRIENT MEDIUM CONTAINING DIFFERENT AMOUNTS OF GROUND POTATO

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	10	0 000 1	0 000 001
Control Potato cylinder medium without crystal violet	3	2	1
"S" (standard glycerol agar broth medium) + 60% ground potato	3	2	0
"S" + 30% ground potato	4	3	2
"S" + 20% ground potato	3	2	2
"S" + 10% ground potato	2	2	1
"S" + 5% ground potato	1	1	0

The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case and in most cases the experiment was duplicated a second time. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation

The findings recorded in Table I make it evident that tubercle bacilli grow best on a medium containing, in addition to the usual ingredients of 5 per cent glycerol broth agar, about 30 per cent ground potato. On this medium a better growth was obtained than on the plain broth potato cylinder medium without crystal violet which would seem to require elucidation in view of the fact that this medium is not selected in the final analysis when choosing a method for isolating tubercle bacilli. The explanation lies in the fact, however, that crystal violet incorporated in the 30 per cent ground potato glycerol broth agar medium exerted a far more deleterious influence on the growth of the tubercle bacilli than that used with the cylinder medium.

b *The Glycerol Concentration in the Medium*—Since in isolating tubercle bacilli, especially when present in small numbers, any slight variation in composition of medium may mean a diminished power of the bacilli to grow, it was deemed advisable to test all the components of the medium including the glycerol. Therefore varying amounts from 1 to 5 per cent were added to a basic medium (B) consisting of ground potato 20 per cent, broth (4x) 13.4 per cent and agar 1.1 per cent. The culture medium was neutralized to litmus by means of a 10 per cent solution of sodium carbonate (c p anhydrous) and it was then sterilized by autoclaving for thirty minutes at 15 pounds pressure. The results of this study are recorded in Table II.

It is to be noted from the data recorded in Table II that with heavier seeding the virulent human tubercle (Gluckson) bacilli grew best on the ground potato broth agar medium containing 4 per cent glycerol while after lighter seeding (0 000 001 mg per cc) the medium containing 2 to 2½ per cent of glycerol proved more suitable.

This experiment was duplicated, using, in place of the human bacilli, a virulent strain of bovine tubercle bacilli and the findings are recorded in Table III

The findings in Table III indicate that virulent bovine tubercle bacilli with heavier seeding (1 mg per cc) grew best in the potato broth agar medium containing 4 per cent glycerol while after seeding with small numbers of bacilli they grew best with a concentration of 20 to 25 per cent of glycerol

TABLE II

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON THE POTATO BROTH AGAR MEDIUM CONTAINING VARYING AMOUNTS OF GLYCEROL

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	10	0 000,1	0 000,001
Control Potato cylinder broth medium without crystal violet	3*	2	1
"B" (potato broth agar medium) + 5% glycerol	3	3	1
"B" + 4% glycerol	4	3	1
"B" + 3% glycerol	3	3	1
"B" + 2½% glycerol	3	3	2
"B" + 2% glycerol	3	3	2
"B" + 1% glycerol	1	2	1

\*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation

TABLE III

THE GROWTH OF VIRULENT BOVINE TUBERCLE BACILLI ON THE POTATO BROTH AGAR MEDIUM CONTAINING VARYING AMOUNTS OF GLYCEROL

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	10	0 000 1	0 000 001
Control Potato cylinder broth medium without crystal violet	2*	1	1
"B" (potato broth agar medium) + 5% glycerol	3	2	1
"B" + 4% glycerol	4	2	1
"B" + 3% glycerol	3	2	1
"B" + 2½% glycerol	3	2	2
"B" + 2% glycerol	3	2	2
"B" + 1% glycerol	2	1	1

\*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation

c *The Broth Concentration in the Medium*—In order to test the effect of the broth concentration in the glycerol potato agar medium upon the growth of tubercle bacilli a basic medium ("M") was prepared containing 20 per cent ground fresh potato, 2 per cent pure glycerol, and 11 per cent agar-agar. To this was added varying amounts from 5 to 3 per cent of 4X beef broth, containing 12 gm of Liebig's Beef Extract, 40 gm of Bacto-peptone, and 20 gm of pure sodium chloride in 1000 cc of tap water. The media after sterilization in the autoclave at 15 pounds for three minutes was

seeded with large and small amounts of virulent human tubercle bacilli and incubated for two months with the results recorded in Table IV

The findings recorded in Table IV indicate that higher concentrations of broth added to glycerol potato agar mediums have a retarding influence upon the growth of the virulent human tubercle bacilli while in the presence of



Fig 1—The effect of the individual components of glycerol broth potato cylinder medium upon the growth of virulent human tubercle bacilli eight weeks after seeding with 0.000.1 mg suspension.

- Tube 1 Glycerol broth potato cylinder medium  
 Tube 2 Broth potato cylinder medium without glycerol  
 Tube 3 Potato cylinder medium with 5 per cent glycerol without broth  
 Tube 4 Potato cylinder medium with 0.9 per cent sodium chloride solution no glycerol or broth  
 Tube 5 Potato cylinder medium without addition of liquids no glycerol or broth

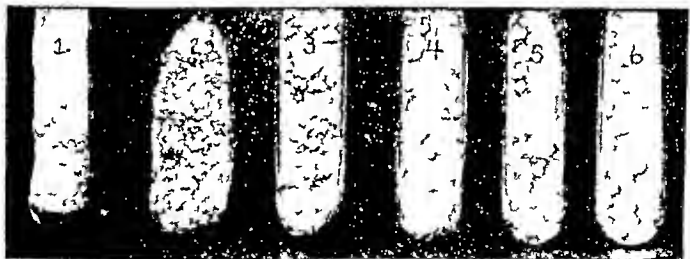


Fig 2—Growth of virulent human tubercle bacilli on medium containing 13.4 per cent 4% broth per cent glycerol 1.1 per cent agar and varying amount of ground potato six weeks after seeding with 0.000.1 mg suspension

- Tube 1 Glycerol broth potato cylinder medium  
 Tube 2 Glycerol broth agar medium A with 60 per cent ground potato  
 Tube 3 A with 30 per cent ground potato  
 Tube 4 A with 0 per cent ground potato  
 Tube 5 A with 10 per cent ground potato  
 Tube 6 A with 5 per cent ground potato

smaller amounts of from 5 to 10 per cent the growth occurred with the same vigor as upon the standard potato cylinder medium without crystal violet

It might justly be asked at this time whether the addition of broth

to the potato medium is necessary, since this was not touched upon in the foregoing studies. This will be given consideration later in the paper after we have presented the result of the study of optimum concentration of crystal violet capable of inhibiting the growth of the undesirable contaminants and still being innocuous to the growth and viability of the tubercle bacilli.

*d The Crystal Violet Concentration in the Medium*—In order to determine the efficiency of various concentrations of crystal violet for inhibiting

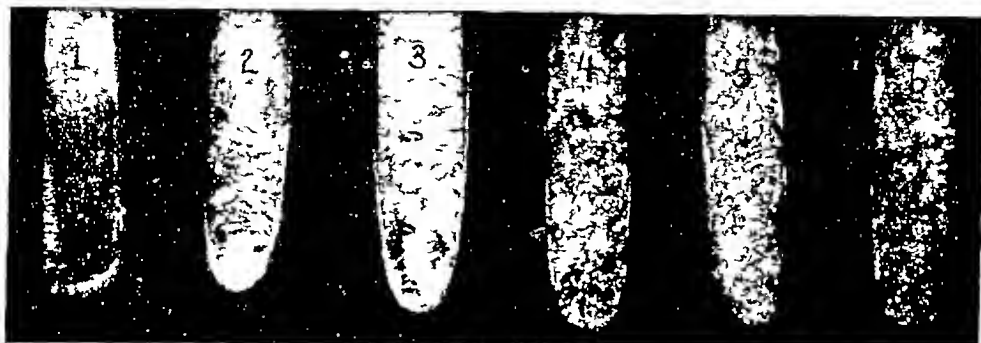


Fig 3—The growth of virulent human tubercle bacilli on broth agar potato medium containing 13.4 per cent 4X broth, 20 per cent ground potato, and 1.1 per cent agar with varying amounts of glycerol three weeks after seeding with 1 mg suspension.

- Tube 1 Glycerol broth potato cylinder medium
- Tube 2 Broth agar potato medium B with 5 per cent glycerol.
- Tube 3 B with 4 per cent glycerol
- Tube 4 B with 3 per cent glycerol
- Tube 5 B with 2 per cent glycerol
- Tube 6 B with 1 per cent glycerol



Fig 4—The growth of virulent human tubercle bacilli on broth agar potato medium containing 13.4 per cent 4X broth, 20 per cent ground potato, and 1.1 per cent agar with varying amounts of glycerol nine weeks after seeding with 0.000 001 mg suspension.

- Tube 1 Glycerol broth potato cylinder medium
- Tube 2 Broth agar potato medium B with 5 per cent glycerol.
- Tube 3 B with 4 per cent glycerol
- Tube 4 B with 3 per cent glycerol
- Tube 5 B with 2 per cent glycerol
- Tube 6 B with 1 per cent glycerol

the growth of the contaminants and permitting the unhampered growth of tubercle bacilli the basic medium ("M") was prepared containing 20 per cent ground fresh potato, 13.5 per cent 4X broth, 2 per cent glycerol, 1.1 per cent agar-agar, and 63.4 per cent tap water. To this medium was added varying amounts of standard crystal violet solution, using a 1 per cent alco-

holic solution for this purpose, so that the medium contained amounts of crystal violet ranging from 0.02 per cent to 0.001 per cent. In order to determine the effect of these mediums upon the growth of tubercle bacilli they were seeded with suspensions of virulent human tubercle bacilli containing from 10 to 0.000,001 mg of bacilli per cubic centimeter of suspension. In Table V are recorded the growth results from such seedings.

The results recorded in Table V indicate clearly that a slightly better growth occurs on the control glycerol potato broth medium without crystal

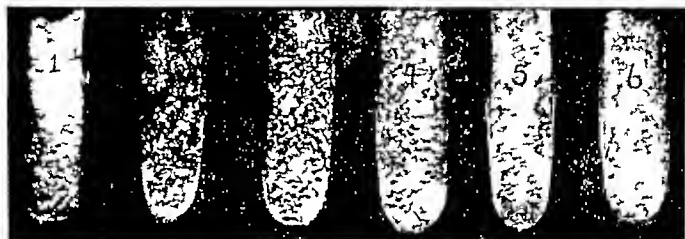


Fig. 5.—The growth of virulent human tubercle bacilli on glycerol agar potato medium containing 2 per cent glycerol 20 per cent ground potato 1.1 per cent agar and varying amounts of 4X normal strength of broth four weeks after seeding with 1 mg suspension.

- Tube 1 Glycerol broth potato cylinder medium
- Tube 2 Glycerol agar ground potato C with 30 per cent 4X broth.
- Tube 3 C with 20 per cent 4X broth.
- Tube 4 C with 13.4 per cent 4X broth.
- Tube 5 C with 10 per cent 4X broth.
- Tube 6 C with 5 per cent 4X broth.

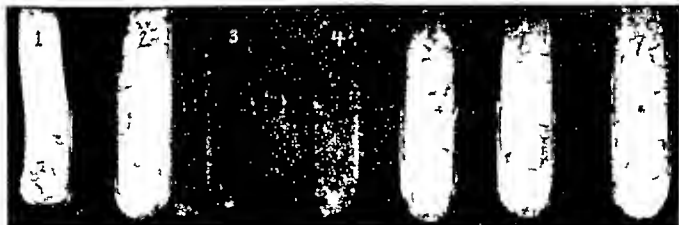


Fig. 6.—The growth of virulent human tubercle bacilli on glycerol agar broth potato medium containing 20 per cent potato 13.4 per cent 4X normal strength broth 1.1 per cent glycerol 1.1 per cent agar with varying amounts of crystal violet, four weeks after seeding with 1 mg suspension.

- Tube 1 Crystal violet glycerol broth potato cylinder medium
- Tube 2 Glycerol agar broth potato medium D without crystal violet.
- Tube 3 D with 0.02 per cent crystal violet.
- Tube 4 D with 0.01 per cent crystal violet.
- Tube 5 D with 0.002 per cent crystal violet.
- Tube 6 D with 0.0015 per cent crystal violet.
- Tube 7 D with 0.001 per cent crystal violet.

violet than it did on the control potato cylinder broth medium without crystal violet whether seeded heavily (10 mg) or lightly (0.000,001 mg) while all the glycerol potato broth mediums containing crystal violet in amounts down to 0.0015 per cent caused a retardation of growth.



For the purpose of testing out the value of these same crystal violet containing potato agar mediums in the isolation of tubercle bacilli from contaminated sources eight positive sputums were treated with an equal volume of 6 per cent sulphuric acid for thirty minutes at incubator temperature and after dilution and centrifugation the sediment was seeded on the mediums. The findings are recorded in summary form in percentages of isolation and contamination in Table VI.

TABLE IV

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GLYCEROL POTATO AGAR MEDIUM CONTAINING VARYING AMOUNTS OF BEEF BROTH

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	10	0 000 1	0 000 001
Potato cylinder medium without crystal violet	3†	2	1
"M" (2% glycerol potato agar medium) + 30% 4X Broth*	1	1	0
"M" + 20% 4X Broth	2	1	1
"M" + 10% 4X Broth	3	2	1
"M" + 5% 4X Broth	3	2	1

\*4X Broth indicates a concentrated broth mixture containing four times the usual amounts of solid constituents as are contained in a commonly used laboratory broth or 4X (3 gm beef extract + 10 gm peptone + 5 gm sodium chloride) in 1000 cc tap water.

†The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation.

TABLE V

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GLYCEROL POTATO BROTH AGAR CONTAINING VARYING AMOUNTS OF STANDARD CRYSTAL VIOLET

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUM		
	10	0 000,1	0 000,001
Control Potato cylinder medium without crystal violet	3*	2	1
"M" (control glycerol potato broth agar without crystal violet)	4	3	2
"M" + 0 02% crystal violet	0	0	0
"M" + 0 01% crystal violet	0	0	0
"M" + 0 002% crystal violet	1	0	0
"M" + 0 0015% crystal violet	2	1	1
"M" + 0 001% crystal violet	4	2	1

\*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation.

The findings recorded in Table VI indicate that crystal violet incorporated in the glycerol potato broth agar medium result in a decreased percentage of tubercle bacillus isolations from the positive sputums as well as an absence of striking improvement in destroying contaminants in serviceable concentration of crystal violet as compared to the standard potato cylinder broth medium containing 0 0015 per cent crystal violet. Petroff's gentian violet egg medium proved superior to the agar mediums but inferior to the potato cylinder medium. There are probably two factors accountable for the inferior quality of the ground potato medium as compared to the cylinder medium, the

greater inhibitory influence of the crystal violet in the agar medium being out and the other possibly being due to the diminished value of the potato as a nutrient medium for the tubercle bacilli when oxidative changes have occurred coincident with a darkening of the freshly ground potatoes which was almost unavoidable in this type of preparation. The possibility that enzymatic (oxidase) oxidation destroyed the important active components of the potato for the growth of tubercle bacilli led to an experiment aiming to destroy the action of these enzymes. Therefore, before preparing the medium the whole unpeeled potato was first autoclaved at 15 pounds pressure for thirty minutes and was then peeled and ground and mixed with glycerol broth agar in the proportions of 30 per cent autoclaved ground potato, 13.5 per cent 4X broth, 2.5 per cent glycerol 10 per cent agar, and 53 per cent tap water to form control medium 'N' and to this was added varying proportions of crystal violet in concentrations from 0.01 to 0.0003 per cent. These mediums supported growth in the proportion revealed by the findings recorded in Table VII.

TABLE VI

ISOLATIONS AND CONTAMINATIONS FROM SPITUMS AFTER EFFICIENTLY SULPHURIC ACID TREATMENT RESULTING ON GROUND POTATO BROTH AGAR MEDIUM CONTAINING VARYING AMOUNTS OF CRYSTAL VIOLET

CULTURE MEDIUM	CULTURE RESULT IN PERCENTAGE	
	POSITIVE ISOLATION OF TUBERCLE BACILLI	CONTAMINATIONS
Control Potato cylinder broth medium + 0.001% crystal violet	66	13
Petroff's gentian violet egg medium	4	13
"M" (control glycerol potato broth agar with out crystal violet)	38	1
"M" + 0.01% crystal violet	0	3
"M" + 0.001% crystal violet	31	13
"M" + 0.0003% crystal violet	31	10

The tube of medium were sealed from each of the eight sputums on the different mediums tested.

TABLE VII

THE GROWTH OF VIRULENT HUMAN TUBERCLE BACILLI ON GROUND POTATO AGAR MEDIUM PREPARED FROM AUTOCLAVED WHOLE POTATOES AND CONTAINING VARYING AMOUNTS OF CRYSTAL VIOLET

CULTURE MEDIUM	AMOUNT OF BACILLI IN 250 PER C. IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	10	0.0001	0.000001
Control Potato cylinder medium without crystal violet	3	2	-
Control Potato cylinder medium with 0.001% crystal violet		2	2
"N" (Control Autoclaved potato agar medium without crystal violet)		2	2
"N" + 0.01% crystal violet	0	0	0
"N" + 0.002% crystal violet	1	1	1
"N" + 0.001% crystal violet	2		2
"N" + 0.0003% crystal violet	3	1	1

The arbitrary grading from 0 to 3 for the amount of growth of the tubercle bacilli on the mediums are not identical to those recorded in the previous Tables I to V but are arbitrarily used to bring out finer differences in this tabulated study and therefore cannot be compared with the graded findings in the previous table. The readings with the 10 mg suspension seedlings were made after four weeks incubation with the 0.0001 mg suspension seedlings after eight weeks and with the 0.000001 mg after twelve weeks.

It is to be noted from the data presented in Table VII that the tubercle bacilli grew about equally well on the two potato cylinder mediums, with and without crystal violet, on the control medium "N" prepared from previously autoclaved whole potatoes, ground and mixed with glycerol broth and agar, and on the latter medium containing 0.0003 per cent crystal violet. The same mediums were, therefore, used for the study of the isolation and contaminations developed from 8 positive tuberculous sputums after preliminary sulphuric acid treatment as in the previous experiment recorded in Table VI. These results are recorded in Table VIII.

It is to be noted from the data recorded in Table VIII that the medium prepared from autoclaved whole potatoes ground and mixed with glycerol broth and agar in suitable proportions and containing 0.0003 per cent crystal violet yielded results in per cent isolations and contaminations about equal to that of potato cylinder broth medium prepared with 0.0015 per cent crystal violet. Greater amounts of dye in this medium were detrimental to growth for isolation purposes.

TABLE VIII

ISOLATIONS AND CONTAMINATIONS FROM SPUTUMS AFTER PRELIMINARY SULPHURIC ACID TREATMENT RESULTING ON POTATO BROTH AGAR MEDIUM, PREPARED FROM AUTOCLAVED POTATOES, CONTAINING VARYING AMOUNTS OF CRYSTAL VIOLET

CULTURE MEDIUM	CULTURE RESULTS IN PERCENTAGE	
	POSITIVE ISOLATIONS FOR TUBERCLE BACILLI	CONTAMINATIONS
Control Petroff's gentian violet egg medium	69*	19
Control Potato cylinder medium with 0.0015% crystal violet	88	13
"N" (Control Autoclaved potato agar medium without crystal violet)	75	28
"N" + 0.01% crystal violet	0	0
"N" + 0.002% crystal violet	66	3
"N" + 0.001% crystal violet	66	19
"N" + 0.0003% crystal violet	84	19

\*Five culture tubes were seeded from each of the eight sputums on the different mediums tested.

*e Growth of Tubercle Bacilli on Potato Without Broth*—In the studies recorded in an earlier part of this paper it was noted that broth added to potato mediums in high concentrations had a deleterious influence upon the growth of the tubercle bacilli but that in low concentrations very little effect was noted upon their growth. This made it appear likely that the addition of broth might not be essential in the potato medium and this is borne out by the culture studies recorded in Table IX.

Since these preliminary experiments indicate that the addition of broth is not essential to obtaining good growth on potato mediums, we have initiated a more extensive study of this with pure suspensions of tubercle bacilli as well as trying isolations from positive sputums. This study has not advanced sufficiently far to report completely at this time but will be reserved for a subsequent complete contribution. However, Table X, records the tests with the potato cylinder and varying amounts of glycerol from 1 to 5 per cent in the 10 to 15 cc of tap water, appear sufficiently conclusive to record at this time.

It is thus to be noted from the data recorded in Table X that the bacilli grew about equally well especially in the high dilutions of seeding regardless whether broth was present in the solution at the bottom of the culture tubes into which the potato cylinders were dipped or whether sterile tap water containing 1 to 5 per cent glycerol was used for this purpose

TABLE IX

THE GROWTH OF TUBERCLE BACILLI ON GROUND POTATO GLYCEROL AGAR MEDIUM IN THE ABSENCE OF BROTH

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	10	0 000 1	0 000 001
Potato cylinder glycerol broth medium without crystal violet	3	~	1
Ground potato glycerol† broth agar medium	4	~	1
Ground potato glycerol‡ agar medium without broth	4	2	1

The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation.

†This medium was prepared from autoclaved whole potatoes which were then mashed and 30 per cent added to 11 per cent agar agar 134 per cent four times normal strength broth, 2 per cent glycerol and 53.5 per cent tap water.

‡This medium was prepared from autoclaved whole potatoes which were mashed and 30 per cent added to 11 per cent agar agar 2 per cent glycerol and 68.9 per cent tap water but no broth.

TABLE X

THE GROWTH OF TUBERCLE BACILLI ON CRYSTAL VIOLET POTATO CYLINDERS DIPPED IN GLYCEROL SOLUTIONS WITHOUT BROTH

CULTURE MEDIUM	AMOUNT OF BACILLI IN MG PER CC IN SUSPENSION USED FOR SEEDING CULTURE MEDIUMS		
	10	0 000 1	0 000,001
Crystal violet potato cylinder in 1 cc .5% glycerol broth	4*	2	1
"P" (Crystal violet potato cylinder) in 1 cc .5% glycerol in tap water	3	2	1
'P' in 1 cc 4% glycerol water	3	2	1
'P' in 1 cc 3% glycerol water	3	2	1
'P' in 1 cc 2% glycerol water	3	2	1
'P' in 1 cc 1% glycerol water	3	2	1

\*The arbitrary grading from 0 to 4 indicates the approximate amount of growth obtained from an average of 10 or more tubes seeded in each case. In the 10 column the readings recorded are after four weeks incubation in the 0 000 1 mg column after eight weeks and in the 0 000 001 mg column after ten weeks incubation.

The experiments in which the isolation of tubercle bacilli from contaminated pathologic specimens was studied using the glycerol tap water crystal violet potato cylinders without broth as culture medium has not progressed sufficiently far to warrant a definite recommendation of this medium, although the foregoing study with suspensions is encouraging.

Although the cooked ground potato crystal violet agar medium gave an efficiency about equal to the crystal violet potato cylinder broth medium, we prefer the crystal violet potato cylinder broth medium until a more exhaustive personal experience has disclosed no objections especially in uniformity of preparation of the cooked ground potato crystal violet agar medium. The details of preparation of this medium will therefore, be reserved for a subsequent brief report at which time further experiences with the tap water glycerol crystal violet potato cylinder medium will be reported.

## PART II

A COMPARISON OF THE NEW SULPHURIC ACID CRYSTAL VIOLET POTATO MEDIUM METHOD WITH PETROFF'S SODIUM HYDROXIDE GENTIAN VIOLET EGG MEDIUM METHOD AND GUINEA PIG INOCULATION FOR THE ISOLATION AND DETECTION OF TUBERCLE BACILLI IN SPUTUMS, URINES AND ANIMAL TISSUES

In order to determine the comparative value of the new sulphuric acid crystal violet potato medium method, Petroff's sodium hydroxide gentian violet egg medium method and the sensitive guinea pig inoculation method for the isolation and detection of tubercle bacilli a series of microscopically positive and negative sputums, as well as urines, were used as test materials and in addition tissues (lung, liver, spleen, bone marrow, and kidney) from intravenously infected dogs and rabbits were submitted to analysis by these three methods

a *Detection of Tubercle Bacilli in Sputums*—In order to compare the efficiency of the sulphuric acid crystal violet potato medium method with Petroff's sodium hydroxide gentian violet egg method on positive sputums, 93 microscopically positive sputums were used as tests and 366 culture tubes were seeded for each of the two methods. The summarized findings in this study are briefly recorded in Table XI. To have done guinea pig inoculation tests in this series would obviously have been unnecessary so they were omitted

TABLE XI

A COMPARISON OF THE SULPHURIC ACID CRYSTAL VIOLET POTATO CYLINDER METHOD AND PETROFF'S SODIUM HYDROXIDE GENTIAN VIOLET EGG METHOD FOR THE ISOLATION OF TUBERCLE BACILLI FROM SPUTUMS MICROSCOPICALLY POSITIVE

METHOD OF ISOLATION USED	NUMBER OF CULTURE TUBES OF A TOTAL OF 366 IN WHICH GROWTH OCCURRED	PERCENTAGE OF TOTAL NUMBER OF TUBES IN WHICH GROWTH OCCURRED	NUMBER OF CULTURE TUBES OF TOTAL 366 THAT WERE CONTAMINATED	PERCENTAGE OF TOTAL TUBES THAT WERE CONTAMINATED	NUMBER OF SPUTUMS OF TOTAL 93 THAT WERE POSITIVE	PERCENTAGE OF TOTAL SPUTUMS THAT WERE POSITIVE
Petroff's sodium hydroxide gentian violet egg medium method	129	35	27	7	41	44.0
Sulphuric acid crystal violet potato cylinder method	265	72	75	20	85	91.4

The findings with Petroff's method practically duplicate those previously recorded by other authors, and it is obvious from the foregoing study and results recorded in Table XI, that the sulphuric acid crystal violet potato cylinder method gives a far greater number and percentage isolation from microscopic positive sputums

In order to compare the efficiency of the two culture methods with guinea pig inoculation on sputums, a series of nine microscopically doubtful sputums from positive cases of pulmonary tuberculosis were used. Petroff's sodium

hydroxide gentian violet egg medium method gave growth in the case of two of these nine sputums, while the sulphuric acid crystal violet potato cylinder method and guinea pig inoculation gave positive findings in agreement in five of the total nine doubtful microscopic sputums. The remaining four sputums were negative by all the above three methods. It is thus to be noted that with doubtful sputums from definite cases, the sulphuric acid potato method was in entire accord with the results of guinea pig inoculation. There is, however, another important point worthy of mention at this time and that is that the guinea pig findings concurred with the sulphuric acid potato method in so far as the early appearance of positive findings only when the local glands were incised and smears examined for tubercle bacilli by staining methods, generalized disease with internal organ and splenic involvement in the guinea

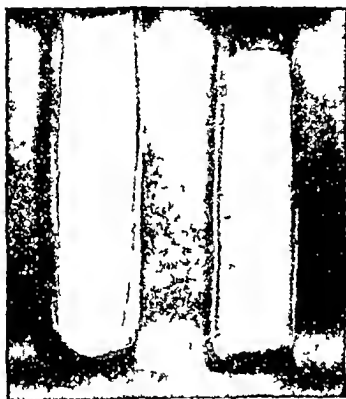


Fig. 7—Isolation of tubercle bacilli from microscopically slightly positive sputum after three weeks incubation by the sodium hydroxide gentian violet egg medium method (tube on left) and by the sulphuric acid crystal violet potato cylinder method (tube on right). There was no growth from this sputum on the gentian violet egg medium even after ten weeks incubation while growth appeared on the potato cylinder medium as early as the third week on all the tubes planted.

pig only develops several weeks later. Frequently smears from the glands are negative for tubercle bacilli in stained preparations and in many of these cases the sulphuric acid potato culture method yielded definite positive growth several weeks before the bacilli were found or definite generalized disease developed in the guinea pig. Petroff's method when positive gave a growth in practically all cases only several or more weeks later than positive evidence developed by the other two methods.

b *Detection of Tubercle Bacilli in Urines*—In order to compare the efficiency of Petroff's method the sulphuric acid crystal violet potato cylinder method and guinea pig inoculation for detecting tubercle bacilli in urines, 24 specimens were collected and tested. Among these were positive and negative microscopic specimens. Of the 24 specimens of urine tested three gave posi

tive cultures by Petroff's method with evident growth occurring only after the fifth week in one sample, and the tenth and thirteenth week respectively in the other two. The sulphuric acid crystal violet potato cylinder method gave evident growth in ten of the twenty-four specimens of urine while guinea pig inoculation gave positive findings in eleven of the twenty-four specimens. The one urine, however, which proved positive by guinea pig inoculation and not by the new culture method was positive only in one guinea pig while a duplicate remained entirely negative for tuberculosis even after three months' observation period. The failure of the culture method in this case may possibly have been due to the absence of tubercle bacilli from the inoculum used for seeding the culture tubes as was the case with the second guinea pig and this must therefore not necessarily be counted against the new culture method for detecting tubercle bacilli.

The earliest evidence for diagnostic purposes was possible with the new potato culture method, as evidenced by macroscopic growth of the tubercle bacilli on the medium, within the fourth or fifth week in the majority of the cases, while by the guinea pig inoculation method a diagnosis could be made in the third or fifth week provided the infected local glands were opened and a smear of the pus examined microscopically for tubercle bacilli. Generalized disease in the guinea pig with involvement of the spleen only occurred several weeks later. The above findings, therefore, indicate that the sulphuric acid crystal violet potato cylinder method is as efficient and as reliable as the guinea pig inoculation method for the detection of tubercle bacilli in sputums and urines.

*c Detection of Tubercle Bacilli in Infected Tissues*—The efficiency of the sulphuric acid crystal violet potato cylinder as compared to Petroff's method and guinea pig inoculation was also borne out in tests with infected animal tissues. For this part of the study 20 dogs and 20 rabbits were given intravenous injections of virulent human tubercle bacilli in varying graded amounts of 10, 0.01, 0.0001, 0.000,001, and 0.000,000,01 mg in fine suspension in sterile saline solution. These animals were then divided into two series, one being killed one week after infection and the second one month after infection. Tissues from five organs, e.g., lung, liver, spleen, kidney, and bone marrow, from each animal were examined. After being ground fine and mixed with sterile 0.9 per cent sodium chloride solution, approximately equal parts were used for the detection of tubercle bacilli by Petroff's method, the sulphuric acid crystal violet potato method and guinea pig inoculation. Specimen results of these studies are recorded in Tables XII, XIII, and XIV chosen at random from a large number of like test experiments with the tissues from rabbits and dogs but showing some significant findings.

The data obtained from the tissue studies in which dog's and rabbit's organs were used as tests and specimens of which are recorded in Tables XII, XIII, and XIV revealed that in general most of the results of guinea pig inoculation were in accord with the findings in the sulphuric acid potato cylinder culture method. At times, however, when the bacilli were sparse in the tissues, due to the small amount given intravenously to the animals,

the sulphuric acid potato method would give positive findings while the guinea pig inoculation method was negative, and vice versa

Since in earlier studies a comparison was made between the crystal violet potato cylinder broth medium and Petroff's gentian violet egg medium which proved the former medium to be far superior for promoting the growth of small numbers of tubercle bacilli seeded thereon than the latter it was also deemed advisable to perform comparative tests of the sodium hydroxide used in Petroff's method for the preliminary treatment of tuberculosis materials and the sulphuric acid used in the sulphuric acid potato method. In addition

TABLE XII

A COMPARISON OF GUINEA PIG INOCULATION AND THE H<sub>2</sub>SO<sub>4</sub>—POTATO CULTURE METHOD WITH PETROFF'S METHOD FOR THE DETECTION OF TUBERCLE BACILLI IN RABBIT TISSUES

TISSUES FROM RABBIT INFECTED INTRAV (1 MONTH) WITH 2 (1 MG VIR. HUMAN TUBERCLE BACILLI	PETROFF'S METHOD	NEW METHOD	GUINEA PIG INOCULATION	
	GROWTH EVIDENCE CONCLUSIVE	GROWTH EVIDENCE CONCLUSIVE	ANTEMORTEM DIAGNOSIS (EVIDENCE NOT CONCLUSIVE)*	POSTMORTEM DIAGNOSIS (EVIDENCE CONCLUSIVE)
	NO OF WEEKS AFTER INOCULATION	NO OF WEEKS AFTER INOCULATION	NO OF WEEKS AFTER INOCULATION	NO OF WEEKS AFTER INOCULATION
	3 4 5 6 7 8 14	3 4 5 6 7 8 14	3 4 5 6 7 8 16	6 16
Bone marrow	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 1 1 + + + +	+
Kidney	0 0 0 0 0 0 0 +	0 1 + + + + + +	0 0 + + + +	+
Liver	0 0 0 0 0 0 0 0	0 0 1 + + + + +	0 1 1 1 1 1 1 +	+
Lung	0 0 0 0 0 0 0 0	0 0 + + + + + +	1 1 + + + +	+(?)†
Spleen	0 0 0 0 0 0 0 0	0 0 0 + + + + +	0 0 1 1 + + + +	+

By antemortem diagnosis is designated the local gland involvement following subcutaneous injection. When the enlargement of the local glands warranted they were incised and examined for the presence of tubercle bacilli and when present the animal was sacrificed within a week or two and examined for generalized disease which is then recorded under Postmortem Diagnosis. Only three gradings are used in these tabulations 0 negative the diagnosis is uncertain or glandular enlargement was insufficient to make a definite diagnosis and + the presence of definite growth of bacilli definite glandular involvement or generalized tuberculosis dependent upon the column under which recorded.

In this guinea pig most of the glands (lingual retroperitoneal and tracheal) were enlarged but no bacilli were found in smears and the spleen and rest of the organs were not tuberculous.

TABLE XIII

A COMPARISON OF GUINEA PIG INOCULATION AND THE H<sub>2</sub>SO<sub>4</sub>—POTATO CULTURE METHOD WITH PETROFF'S METHOD FOR THE DETECTION OF TUBERCLE BACILLI IN RABBIT TISSUES

TISSUES FROM RABBIT INFECTED INTRAV (1 MONTH) WITH 0.01 MG VIR. HUMAN TUBERCLE BACILLI	PETROFF'S METHOD	NEW METHOD	GUINEA PIG INOCULATION	
	GROWTH EVIDENCE CONCLUSIVE	GROWTH EVIDENCE CONCLUSIVE	ANTEMORTEM DIAGNOSIS (EVIDENCE NOT CONCLUSIVE)	POSTMORTEM DIAGNOSIS (EVIDENCE CONCLUSIVE)
	NO OF WEEKS	NO OF WEEKS	NO OF WEEKS	NO OF WEEKS
	3 4 5 6 7 8 14	3 4 5 6 7 8 14	3 4 5 6 7 8 16	6 16
Bone marrow	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0
Kidney	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0	0
Liver	0 0 0 0 0 0 + +	0 0 0 0 0 0 + +	0 0 0 0 0 0 0 0	0
Lung	0 0 0 0 0 0 + +	0 0 0 + + + + +	0 0 + + + +	+
Spleen	0 0 0 0 0 0 0 0	0 0 0 + + + + +	1 1 + + + + + +	+

By antemortem diagnosis is designated the local gland involvement following subcutaneous injection. When the enlargement of the local glands warranted they were incised and examined for the presence of tubercle bacilli and when present the animal was sacrificed within a week or two and examined for generalized disease which is then recorded under Postmortem Diagnosis. Only three gradings are used in these tabulations 0 negative the diagnosis is uncertain or glandular enlargement was insufficient to make a definite diagnosis and + the presence of definite growth of the bacilli definite glandular involvement or generalized tuberculosis dependent upon the column under which recorded.



it was considered advisable to determine whether a residence on Petroff's egg medium as well as the crystal violet potato cylinder may not exert a detrimental influence upon the bacilli from tuberculous tissues planted on these mediums. The results of these tests using the guinea pig as test animal for determining the viability of the bacilli is recorded in Table XV.

It is clearly evident from the data recorded in Table XV that both the sodium hydroxide treatment and the culturing of the prepared plant on the gentian violet egg medium as prescribed for Petroff's method have a slight but definite detrimental influence upon the viability of small numbers of tubercle bacilli contained in both the spleen of the rabbit and the liver of the dog as determined by guinea pig inoculation, which injurious effect upon

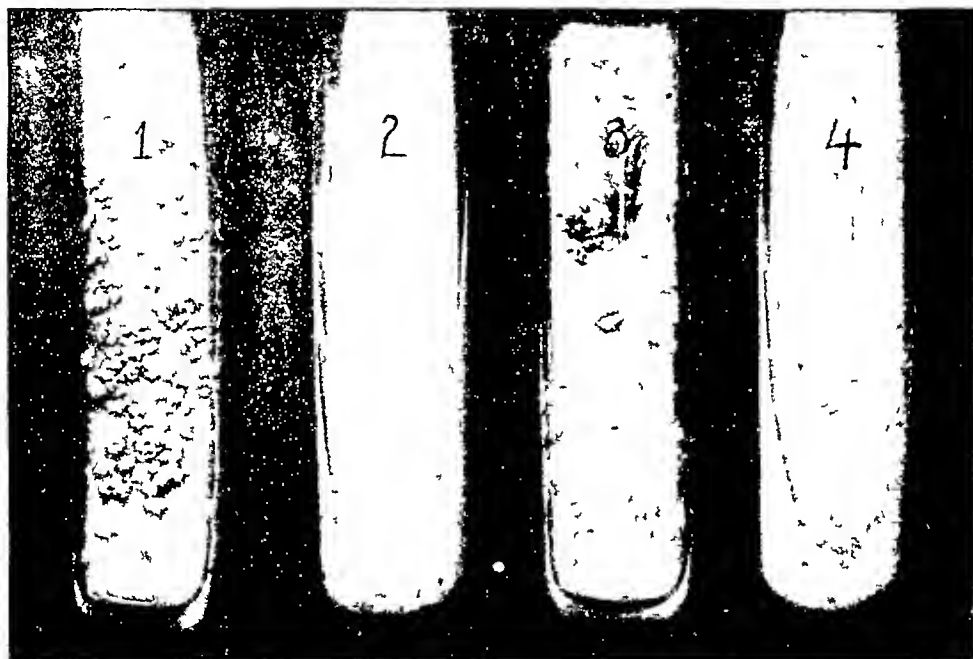


Fig. 8—A comparison of cultures from the spleen of rabbits intravenously infected with virulent human tubercle bacilli eight weeks after isolation by Petroff's method (Tubes 2 and 4) and by the sulphuric acid crystal violet potato cylinder method (Tubes 1 and 3). The isolation in Tubes 1 and 2 was from a rabbit given an intravenous injection of 1 mg. of bacilli and in Tubes 3 and 4 0.01 mg. Tubes 1 and 3 revealed growth as early as the third week while Tubes 2 and 4 were still negative after twelve weeks incubation.

the bacilli was not evident after the sulphuric acid treatment and seeding upon the crystal violet potato cylinder broth medium. These findings account for the greater delicacy of the sulphuric acid potato method as compared to the sodium hydroxide gentian violet egg method as well as for the higher percentage isolations from sputums, urines, and animal tissues obtained with the former method. These data, recorded in Table XV, also again emphasize the efficacy of the sulphuric acid potato method for isolating tubercle bacilli from materials infected with tubercle bacilli and its equality in percentage isolations to the guinea pig over which it has many advantages especially in convenience of examination and maintenance.

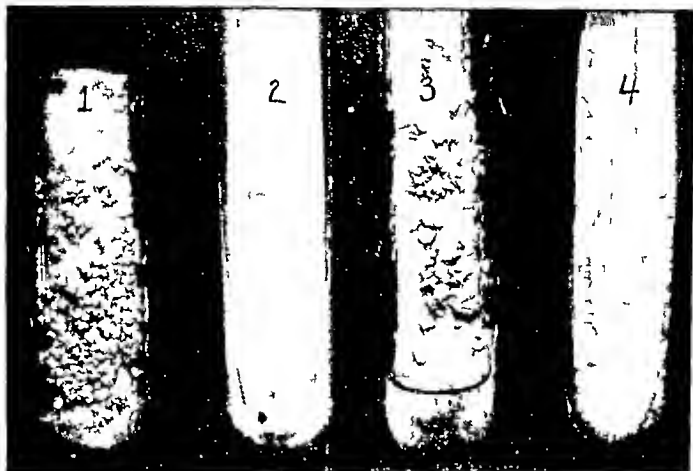


Fig 9—A comparison of cultures from the liver of dogs intravenously infected with virulent human tubercle bacilli eight weeks after isolation by Petroff's method (Tubes 2 and 4) and by the sulphuric acid crystal violet potato cylinder method (Tubes 1 and 3). The isolation in Tubes 1 and 2 was from a dog given an intravenous injection of 1 mg of bacilli and in Tubes 3 and 4 0.01 mg. Tubes 1 and 3 revealed growth as early as the third week while Tube 2 revealed a sparse growth at eight weeks as shown above and Tube 4 was negative to the twelfth week.

TABLE XIV

A COMPARISON OF GUINEA PIG INOCULATION AND THE H SO<sub>4</sub>—POTATO CULTURE METHOD WITH PETROFF'S METHOD FOR THE DETECTION OF TUBERCLE BACILLI IN DOG TISSUES

TISSUES FROM DOG INFECTED INTRAV (1 MONTH) WITH 0.0001 MG VIR HUMAN TUBERCLE BACILLI	PETROFF'S METHOD										NEW METHOD										GUINEA PIG INOCULATION																			
	GROWTH EVIDENCE CONCLUSIVE										GROWTH EVIDENCE CONCLUSIVE										ANTEMORTEM DIAGNOSIS (EVIDENCE NOT CONCLUSIVE)										POSTMORTEM DIAGNOSIS (EVIDENCE CONCLUSIVE)									
																					NO OF WEEKS										NO OF WEEK									
	2	3	4	6	7	8	14	2	3	4	5	6	7	8	14	2	3	4	5	6	7	8	16	6	16															
Bone marrow	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
Kidney	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
Liver	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	0	+																
Lung	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+																
Spleen	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+																

By antemortem diagnosis is designed the local gland involvement following subcutaneous injection. When the enlargement of the local glands warranted they were incised and examined for the presence of tubercle bacilli and when present the animal was sacrificed within a week or two and examined for generalized disease which is then recorded under Postmortem Diagnosis. Only three gradings are used in these tabulations 0 negative, ? the diagnosis is uncertain or glandular enlargement was insufficient to make a definite diagnosis and + the presence of definite growth, definite glandular involvement or generalized tuberculosis dependent upon the column under which recorded.

†Although this animal revealed a slight nodular lesion in the abdominal wall after the eighth to tenth week there developed no enlargement of the local glands and at postmortem after four months there was found only a slight splenic involvement with several small tubercles but no enlargement of this organ and no involvement of the other organs. The retroperitoneal and tracheal glands were also slightly enlarged but not significantly so.

TABLE XV

THE EFFECT OF THE PRELIMINARY TREATMENT WITH SODIUM HYDROXIDE OR SULPHURIC ACID, AND PLANTING ON GENTIAN VIOLET EGG MEDIUM OR CRYSTAL VIOLET POTATO MEDIUM ON THE VIABILITY OF TUBERCLE BACILLI CONTAINED IN TISSUES

EXPERIMENT	TISSUE TESTED																
	SILLEN OF RABBIT GIVEN INTRAVENOUS INJECTION 1 WEEK BEFORE EXAMINATION							LAYER OF DOG GIVEN INTRAVENOUS INJECTION 1 WEEK BEFORE EXAMINATION									
	0.01 MG							0.01 MG									
	10 MG							10 MG									
	2	4	6	8	12	2	4	6	8	12	2	4	6	8	12		
Control guinea pig inoculated with tissue direct	0*	±	+	+	+2	+	+	+	+		0	+	+	+	+		
Guinea pig inoculated with tissue treated with H <sub>2</sub> SO <sub>4</sub> alone	0	+	+	+		±	+	+	+		±	+	+	+	+		
Guinea pig inoculated with tissue treated with H <sub>2</sub> SO <sub>4</sub> and in cubated for 3 weeks on potato medium	++	++	+	+		++	++	++	+		+	++	++	++	++		
Culture results after H <sub>2</sub> SO <sub>4</sub> treatment and planting on potato cylinder medium	0	1†	2	3	4	0	2	3	4	4	±	1	2	4	4	4	4
Guinea pig inoculation with tissue treated with NaOH (Petroff's alone)	0	+	+	+	+	±	+	+	1		0	+	+	+	+		
Guinea pig inoculated with tissue treated with NaOH and in cubated for 3 weeks on Petroff's egg medium	0	0	0	0	0	0	±	+	++	1	0	0	0	0	0	++	3
Culture results after NaOH treatment and planting on Petroff's egg medium	0	0	0	0	0	0	0	0	0	0	0	0	0	1†	1	0	2§

\*The tuberculous tissues from the dogs and rabbits were used one week after intravenous injection of a strain of virulent human tubercle bacilli. The tuberculous involvement of the local site of subcutaneous inoculation and local glands is graded from 0 to +++ according to the grade of palpable enlargement. The numeral accompanying the last grading recorded indicates that at postmortem after the number of weeks under which column the figure is given the animal presented either 0 no tuberculous involvement 1 enlargement of the local glands alone 2 enlargement of the local and tributary glands and slight involvement of the spleen 3 generalized glandular enlargement marked splenic involvement and enlargement and slight hepatic and pulmonary involvement and 4 a marked generalized involvement of most organs with marked splenic enlargement and involvement. A 'u' after the local gland grading indicates that the local lesion has ulcerated and is discharging. The guinea pig readings recorded in each case are the average for two animals inoculated for each tissue tested or for separate animals inoculated from two separate culture tubes. If the reading recorded under the culture results is the average of at least two culture tubes seeded and usually of 3 or 4 culture tubes. The amount of growth is graded from 0 no macroscopic evident growth to 4 a profuse thick growth covering most of the medium.

†Growth was obtained in this case on only one of three culture tubes.

‡Growth was obtained in this case on two of three culture tubes.

## SUMMARY AND CONCLUSIONS

For the isolation of tubercle bacilli from contaminated tuberculous materials, such as urines, sputums, and tissues, the sulphuric acid crystal violet potato cylinder method is superior to the sodium hydroxide gentian violet egg medium method and equal in efficiency to the guinea pig inoculation test. Small numbers of tubercle bacilli in tissues (dog's liver and rabbit's spleen) are not appreciably affected by the preliminary treatment with an equal volume of 6 per cent sulphuric acid at 37° C for thirty minutes used to destroy the contaminants nor by planting on the crystal violet potato cylinder medium while an equal volume of 3 per cent sodium hydroxide for twenty minutes at 37° C has a slight detrimental influence upon the tubercle bacilli which is further enhanced by seeding on gentian violet egg medium as prescribed in Petroff's method.

Ground fresh potatoes suffer changes during grinding and exposure to air which makes them less suitable in the preparation of glycerol agar mediums for growing tubercle bacilli than like mediums made from potatoes ground and mixed with the medium after having been autoclaved for thirty minutes at 15 pounds pressure. In the preparation of the crystal violet potato cylinder medium the amount of glycerol from 1 per cent to 5 per cent, in the broth or tap water used, has no appreciable influence upon the growth of the tubercle bacilli on this medium. It appears from preliminary studies that tap water containing the glycerol can be substituted for the glycerol broth in the preparation of the medium. Likewise it appears that a glycerol crystal violet potato agar medium in which the potatoes were autoclaved before being ground may be substituted for the crystal violet potato cylinder broth medium used for isolating tubercle bacilli. This is being more exhaustively studied. The glycerol content in this medium however, requires to be about 2 to 2½ per cent. If broth is used in the preparation of this medium it must not be used in excess. The most favorable concentration of crystal violet added to this medium neutralized with sodium carbonate solution is about 0.0003 per cent, whereas with the potato cylinder medium the cylinders are best dipped in 0.0015 per cent dye containing 1 per cent sodium carbonate.

The sulphuric acid crystal violet potato method for isolating and detecting tubercle bacilli possesses many advantages over guinea pig inoculation including the possibility of repeated observations economy of performance availability of a culture, when positive for determining the type of bacilli present as well as adding a feeling of confidence and certitude in the findings.

## REFERENCES

- 1 Corper H J, and Uyei Nao. The Isolation of Tubercle Bacilli From Contaminated Tuberculous Material, *Am Rev Tuberc* 16 290-322 1927, *ibid* 9 115-130 1927. The Cultivation of Tubercle Bacilli. An Improved Method for Isolation From Tuberculous Materials. *J LAB & CLIN MED* 13 469-480, 1928.
- 2 Petroff S A. A New and Rapid Method for the Isolation and Cultivation of Tubercle Bacilli Directly From the Sputum and Fees. *J Exper Med* 21 38-42 1915. Some Cultural Studies on Tubercle Bacilli. *Bull Johns Hopkins Hosp* 26 276-29 1915.
- 3 Corper H J, Fiala, L and Kallen L. The Routine Cultivation of Tubercle Bacilli From the Sputum by Petroff's Method. *J Infect Dis* 23 267-274 1918.

TABLE XV

THE EFFECT OF THE PRELIMINARY TREATMENT WITH SODIUM HYDROXIDE OR SULPHURIC ACID, AND PLANTING ON GENTIAN VIOLET Egg MEDIUM OR CRYSTAL VIOLET POTATO MEDIUM ON THE VIABILITY OF TUBERCLE BACILLI CONTAINED IN TISSUES

EXPERIMENT	TISSUE TESTED															
	SILLEN OF RABBIT GIVEN INTRAVENOUS INJECTION 1 WEEK BEFORE EXAMINATION								LIVER OF DOG GIVEN INTRAVENOUS INJECTION 1 WEEK BEFORE EXAMINATION							
	0.01 MG				1.0 MG				0.01 MG				1.0 MG			
	INTERVAL IN WEEKS FOLLOWING SEEDING OF TUBES OR INJECTION OF GUINEA PIG								TUBES OR INJECTION OF GUINEA PIG							
	-	4	6	8	12	2	4	6	8	12	2	4	6	8	12	
Control guinea pig inoculated with tissue direct	0*	±	+	+	+2	+	+++	3			0	+	2	+++		
Guinea pig inoculated with tissue treated with H <sub>2</sub> SO <sub>4</sub> alone	0	+	++			±	+++	3			±	+	++	++		
Guinea pig inoculated with tissue treated with H <sub>2</sub> SO <sub>4</sub> and incubated for 3 weeks on potato medium	++	++	+	+		++	++	++	+		+	++	++	++	++	
Culture results after H <sub>2</sub> SO <sub>4</sub> treatment and planting on potato cylinder medium	0	1†	2	3	4	0	2	3	4	4	±	1	2	4	4	
Guinea pig inoculation with tissue treated with NaOH (Petroff's alone)	0	+	+	+	+	±	+	+	1		0	+	+	++		
Guinea pig inoculated with tissue treated with NaOH and incubated for 3 weeks on Petroff's egg medium	0	0	0	0	0	0	±	+	++	1	0	0	0	0	0	
Culture results after NaOH treatment and planting on Petroff's egg medium	0	0	0	0	0	0	0	0	0	0	0	0	0	1†	1	

\*The infected tissues from the dogs and rabbits were used one week after intravenous injection of a strain of virulent human tubercle bacilli. The tuberculous involvement of the local site of subcutaneous inoculation and local glands is graded from 0 to +++ according to the grade of palpable enlargement. The numeral accompanying the last grading recorded indicates that at postmortem after the number of weeks under which column the figure is given the animal presented either 0 no tuberculous involvement 1 enlargement of the local glands alone 2 enlargement of the local and tributary glands and slight involvement of the spleen 3 generalized glandular enlargement marked splenic involvement and enlargement and slight hepatic and pulmonary involvement and 4 a marked generalized involvement of most organs with marked splenic enlargement and involvement. A 'u' after the local gland grading indicates that the local lesion has ulcerated and is discharging. The guinea pig readings recorded in each case are the average for two animals inoculated for each tissue tested or for separate animals inoculated from two separate culture tubes if the bacilli had been seeded on these before inoculation. The average of at least two culture tubes seeded and usually of 3 or 4 culture tubes. The amount of growth is graded from 0 no macroscopic evident growth to 4 a profuse thick growth covering most of the medium.

†Growth was obtained in this case on only one of three culture tubes.

‡Growth was obtained in this case on two of three culture tubes.

## TUBERCULOUS ENDOMETRITIS\*

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ACCORDING to most textbooks, and authors who have reviewed it, tuberculosis of the female genital tract is of rather common occurrence. Yet a review of the literature on the subject reveals very few specific cases and a rather scant discussion of the subject in general. While this does not justify the conclusion that female genital tuberculosis is rare, from the number of cases we have encountered in our own laboratories, we are of the opinion that it is not as common as is often assumed.

Statistics vary considerably, but none indicate any great frequency in the general run of clinical material. Thus Frank<sup>1</sup> cites Fromme and Heynemann who in 17,470 autopsies found genital tuberculosis in 142 or about 0.81 per cent. Schlumpert, in 3514 autopsies of women and children with tuberculosis found 34 per cent affected with genital lesions. Naturally the incidence of infection must be greater in cases of active tuberculosis than in the general run of clinical and autopsy material, and when we consider that the tubes are most frequently infected, we see at once that tuberculosis of the uterus is not common.

The assumption that primary tuberculous infection occurs in the female genital tract has always been very attractive, especially in view of the manifest portal of entry. But definite evidence of such a mode of infection has never been produced. Such evidence as has been brought forward in support of this theory has been purely circumstantial. Thus Bauereisen<sup>2</sup> cites an autopsy upon the widow of a phthisical man in which tuberculous endometritis was the only lesion of tuberculosis found. The primary lesion of tuberculosis is not necessarily a macroscopic lesion, and the bacilli may gain access to the body and be widely disseminated without any definite macroscopic evidence of the original portal of infection. One should not assert that primary infection of the female genital tract may occur upon circumstantial evidence.

Many lesions of tuberculosis that were formerly considered primary lesions, in the light of more recent experimental investigations appear to be secondary lesions. The pathology of a primary focus of infection is different from that of a secondary whether this infection be endogenous or exogenous. The primary focus is small, discrete, sharply defined, composed almost exclusively of epithelioid cells, and usually shows coexistent involvement of the regional lymph nodes, whereas the secondary infection is greater in area, less sharply defined, variable in component elements and does not show the same tendency to heal that is manifest in the primary focus.

Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9 and 11, 1913.

From the Department of Pathology, The Ohio State University, Columbus, Ohio.

If most tuberculous infections are secondary, the question arises as to the mode of infection of the female genital tract. Direct extension from an adjacent lesion such as a tuberculous peritonitis is possible, but it is probable that with a coexistence of these two lesions the original extension was from the female genital tract to the peritoneum rather than in the opposite direction since the concomitant peritonitis is frequently localized in the pelvis, and intestines and mesenteric lymph nodes are often unaffected. Infection in the majority of cases is metastatic and by the blood stream and the original focus is most frequently in the lungs or bronchial lymph nodes. Such an original focus also bespeaks the infrequency of lymphatic metastasis to the female genital tract. Metastatic extensions in cases of manifest pulmonary tuberculosis are far more widely disseminated than macroscopic examination reveals. Such disseminated foci are only revealed by extensive and careful microscopic examination of all organs. Even then the lesions do not always exhibit the typical textbook form, and special staining of the tubercle bacilli may be required to demonstrate their nature. This indicates that dissemination of tubercle bacilli are frequent and that the development of a macroscopic lesion in organs is rare in proportion to the number of individuals who are infected with tuberculosis, and are manifested in miliary tuberculosis or isolated tuberculosis of some organ or system. Some organs are affected more frequently than others, this has led to the assumption that there is a difference in the resistance of organs to tuberculous infection. There is a difference in resistance but probably not so much an inherent difference in the soil as in the physiologic activities of the particular organ. Thus Krause (quoted by Baldwin, Petroff and Gardner<sup>4</sup>) has suggested that in organs constantly in motion the lymph flow is so active that the bacilli are not allowed to remain in one place sufficiently long for the development of a lesion. In the uterus we not only have an organ which is contracting periodically, but a congestion and desquamation of the entire endometrium, which practically amounts to a physiologic excision of the tuberculous area. Cases of tuberculous endometritis cured by curettage have been reported. The normal endometrium performs a physiologic curettage periodically. We know this prevents the establishment of chronic foci of infection in the endometrium with the common bacteria, and we believe it to be a factor in the prevention of the establishment of chronic tuberculous foci. If this be true, we should expect to find evidence of some primary process that might alter this function along with tuberculous infections, and such is true both in our own cases and in those reported by others. The frequency of tuberculosis in the placenta, following multiple pregnancies, in association with leiomyomas, fibrosis uteri, and even carcinoma, supports this view. Thus tuberculosis is a secondary and complicating lesion in these conditions and not a primary lesion as is usually assumed.

The macroscopic appearance of tuberculous tubes and uteri in 75 per cent of the cases cannot be distinguished from those due to other infecting organisms. A varying number of cases associated with a general tuberculous peritonitis, do not represent true genital tuberculosis, but are a part of another process. In these cases the sago-like tubercles on the surface of the tube and mesosalpinx are really a part of a peritonitis and frequently there is no evidence

of tuberculosis in the genital tract itself. The true genital tuberculosis with which we are concerned is the type with definite lesions in the mucosa or muscularis of the tubes or uterus and in this type there is frequently no characteristic to identify the process on gross examination, and it is these cases that are frequently assigned to some other etiologic agent, especially when routine microscopic examination is not made of every tube or uterus that is removed.

Clinically there is no pathognomonic symptom or even suggestive symptom associated with tuberculous infection of the female genital tract. In the tubes the infection is most frequently assigned to gonococcus or puerperal infection according to the history. In the uterus, a diagnosis of malignancy, adenomyoma, fibromyoma or gonococcus infection is usually made, according to the history and symptoms. In the cervix the differentiation between malignancy and tuberculosis most frequently leans toward malignancy and indeed the differentiation is difficult with the gross specimen and may give rise to no little concern in the microscopic section.

The age of occurrence is of interest in this connection. White<sup>8</sup> found approximately 80 per cent occurring between the ages of sixteen and thirty-six. In general it may be said that pelvic infection of obscure cause and with atypical symptoms between sixteen and thirty-six years of age is always suggestive of tuberculosis. Clinical search for a primary focus of tuberculosis often reveals none. Curettement of the uterus and microscopic examination may yield tubercles, or may be so atypical that the diagnosis is confusing. The infrequency of tuberculous endometritis, and when it does occur the frequent association with other pathologic lesions, especially adenomyoma or fibroma, suggests that a primary pathologic alteration of the physiology of the body of the uterus is a factor in tuberculous infection. Such alterations most manifestly disturb the muscular physiology, and thus the lymphatic drainage.

#### CASE REPORT

L. R., aged thirty, Afro-Caribbean American, was admitted to the hospital with a complaint of "pain in the left side." She was married at the age of thirteen years, had had 3 normal pregnancies in 1910, 1912 and 1914 respectively. The onset of pain was approximately twelve years ago, in 1916 about two years after her last pregnancy. She was eighteen years of age at that time. The only other symptom was a marked leucorrhea. Catamenia was regular but profuse. The physical examination was negative with the exception of definite tenderness in the left lower quadrant. The blood count was not significant and the Wassermann was negative. The x-ray examination of the chest was negative. Pelvic examination was negative except for a rather definite tenderness of the left adnexa. A tentative diagnosis of gonorrheal salpingitis was made and a panhysterectomy performed. The uterus was enlarged and boggy and contained subserous and intramural fibroids. The right tube was bound to the ovary, forming a tubo-ovarian mass. Extensive omental adhesions bound the pelvic viscera in a mass. There was no evidence of tubercles on the serous surface of either ovaries, tubes or the uterus. The endometrium was polypoid.



Microscopic examination revealed numerous tubercles scattered throughout the endometrium and tubal mucosa and the ovaries. The tubercles were of the giant cell type, with many epithelioid cells. There was little tendency toward caseation, coalescence, or conglomerate tubercle formation, and the size of the tubercles was uniform. Complete encapsulation was lacking, although the process did not appear to be very active. The tubercles were scattered throughout the endometrium, and pathologically they appeared to be of recent origin. The associated fibromyomas and fibrosis uteri were significant. There was no invasion of the myometrium.

This case appeared to be one of subinvolution, with multiple fibroids, and a resulting polypoid endometrium upon which subsequently appeared a tuberculous infection. The tuberculous process was certainly not of twelve years duration, which was the period over which the patient had exhibited symptoms. The tuberculous process appeared to be of rather recent origin, while the subinvolution and fibroids were the older process, and were undoubtedly the cause of her earlier symptoms. No evidence of a primary focus was found clinically, which of course does not eliminate such a focus. We believe the infection of both tubes and endometrium was metastatic, probably from a focus somewhere in the respiratory tract.

A second case presented a uterus that was slightly enlarged, and markedly fibrosed. On cut section the body of the uterus was dotted with small definite discrete yellow caseous nodules, about 1 mm in diameter. There were none in the endometrium which was compressed by the thickening of the wall of the uterus. Microscopic examination revealed a marked fibrosis uteri, the nodules were caseous but definitely encapsulated by the fibrosis of the uterus. There were also small tubercles present of the giant cell type, with many epithelioid cells without encapsulation or caseation. The endometrium was atrophic and many microscopic sections revealed only one definite tubercle at the junction of endometrium and myometrium. The tubercles in the myometrium were at varying ages, some being early and others old, large, and caseous.

The sequence in this case seems to have been a subinvolution, fibrosis uteri, and tuberculous infection, with extension of the process to other parts of the uterus.

#### SUMMARY

- 1 There is a marked variation in the reported frequency of tuberculous lesions in the female genital tract.
- 2 The physiologic activities of various portions of the female genital tract may be responsible for variations in resistance to tuberculous infection.
- 3 Tuberculosis of the uterus is more frequently superimposed upon a pathologic than a normal organ.

#### REFERENCES

- 1 Frank, R. T. *Gynecological and Obstetrical Pathology*, New York, D. Appleton and Co., pp. 536, 1922.
- 2 Schlimpert, H. *Die Tuberkulose bei der Frau etc.*, Arch. f. Gynak. 94: 863-925, 1911.
- 3 Bauereisen, A. *Experimenteller Beitrag zur Kohabitationstuberkulose der Weiblichen Genitalien*, Zentralbl. f. Gynak. 44: 905-914, 1920.
- 4 Baldwin, E. R., Petroff, S. A., and Gardner, L. S. *Tuberculosis. Bacteriology, Pathology and Laboratory Diagnosis*, Philadelphia, Lea and Febiger, pp. 342, 1927.
- 5 White, C. *Eden and Lockyer's New System of Gynecology*, London, Macmillan & Co. 1: 611, 1917.

## THE CHEMISTRY AND CYTOLOGY OF SEROUS FLUIDS\*

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VERY little information regarding the amounts of various chemical constituents in serous effusions is found in reviewing recent literature since the advent of modern methods of blood and serum chemistry. Most of the articles available give data on only a few of the chemical substances present. No report on a complete study of a large series of fluids, comparable to information on chemical constituents of the blood in health and disease, is available. For a general summary including relatively old and also recent literature, the reader is referred to Wells<sup>1</sup> who presents a good critical review and bibliography.

Recently Golecki<sup>2</sup> after examination of fairly large numbers of fluids for protein and various nonprotein constituents found that the proteins were high in exudates and low in transudates. Fatty acids were consistently higher in exudates and cholesterol moderately higher in exudates than in transudates. Sugar values were about the same as in blood in both types of serous fluids.

Bezaneon et al.<sup>3</sup> found slightly more chlorides in both transudates and exudates than in blood, whereas calcium values were practically the same as in blood. Fatty substances varied roughly with the protein content, which was increased in inflammatory exudates. The fibrin content was also highest in inflammations. Cholesterol was uniformly moderately higher in exudates than in transudates.

Bernhard<sup>4</sup> found on examining 14 fluids that the amount of nonprotein nitrogenous bodies and sugar content were comparable to the findings in blood. Nathan<sup>5</sup> states that the sugar content is slightly lower in inflammatory fluids than in transudates, a point denied by others including Bezaneon. Orsi<sup>6</sup> found that determinations of glucose were not of differential value in exudates and transudates.

A great deal has been written about the cytodiagnosis of fluids especially by French authors. The best resume of the subject in English is found in Sahli's textbook<sup>7</sup> in which a critical discussion of Widal's original claims is presented. Briefly, Sahli's conclusions are that a sediment showing merely all lymphocytes suggests tuberculosis as the etiology of the effusion but that fluids from other causes may show the same picture. He maintains that the predominance of one type of cell depends less on the primary etiology than on the stage or severity of the process. He advises hesitation in the diagnosis of tumor cells from smears alone, because of the danger of confusion with degenerated cells of other types, in which Bock<sup>8</sup> and Zemansky<sup>9</sup> agree, both

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entirely satisfactory when either was used. Prompt handling of the fluids obviated the use of these chemicals except in a few cases.

The chemical examinations were done by methods ordinarily used for blood and serum chemistry.

On analyzing the findings in the chest fluids (Table I) the specific gravity of the inflammatory exudates is seen to be much higher than that of the transudates in the cardiac congestive cases, varying from 1.019 to 1.025 in the tuberculosis cases (with an average of 1.0215), and 1.022 in the nontuberculosis inflammations compared with 1.012 to 1.014 (average 1.013) in the transudates. The tumor cases likewise were high, 1.018 to 1.025 (average 1.021).

The Rivalta test was consistently marked in the inflammations, most in tuberculosis or tumor, less in nontuberculous inflammations and very scant or negative in the transudates. Spontaneous clotting was most marked in the tuberculous cases, nearly as prominent in those with tumor, moderate in nontuberculous inflammations and only slight or not at all in the transudates.

Analysis of the chemical findings showed the protein content to be practically proportional to the specific gravity of the fluids. The exudates showed much higher albumin, globulin, and fibrin content than the transudates, averaging over three times the values in the latter class of fluids. Albumin-globulin ratios were not constant in any of the groups of fluids. Nonprotein nitrogen, urea, uric acid, creatinine, and chlorides showed values quite comparable to those of the blood. Many simultaneous determinations of these substances in the blood were nearly the same as those in the fluids, indicating free diffusion of these substances through the pleural wall into the chest cavity. The high figures for uric acid in the tumor cases possibly is due to the increased destruction of nucleoprotein.

The sugar content also paralleled the blood sugar quite constantly. In the cardiac group one fluid from a diabetic showed 328 mg. sugar, approximating the amount found in the blood.

Cholesterol values varied considerably in the different fluids with no data of differential value in any group of cases. The same applies to lecithin. Inorganic phosphorus and calcium values were practically the same in all types of fluids and were quite comparable to the amount of the same substances in blood.

Study of the cellular elements in the sediments as well as the fluids in the gross revealed blood in various amounts in all of the fluids. Most was seen in the tumor fluids, less in the tuberculosis cases, and least, amounting to microscopic blood only, in the nontuberculous inflammations. Occasional fluid showed a considerable amount of blood from trauma by the aspirating needle and no doubt traces seen in other fluids could be ascribed to this source.

Studies of the white cells in the tuberculosis fluids showed a marked preponderance of lymphocytes in all but 3 fluids, in which a considerable percentage of polymorphonuclears was seen. All three cases were very acute in onset and severity. The transudates showed characteristically serosal cells singly and in clumps, but in 2 cases with long-standing effusion a high percentage of lymphocytes occurred. Also, over 95 per cent lymphocytes were seen in one case of chronic postpneumonic, nontuberculous pleurisy and two of

lymphosarcoma of the chest (Fig 1) In 4 other nontuberculous pleurisy fluids a high percentage of polymorphonuclears was present along with large numbers of large mononucleated cells, some of which were definitely macrophages showing phagocytosis

All of the several fluids from cases with carcinomatous involvement of the pleura showed masses of large cells, irregular in size and shape, often vacuolated, and sometimes with mitotic figures (Fig 2) Sections of imbedded sediment revealed small fragments of tumor tissue especially gland acini in adenocarcinoma (Figs 3 and 4)

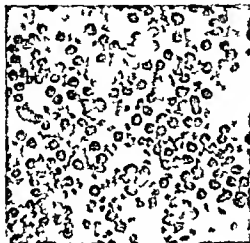


Fig 1

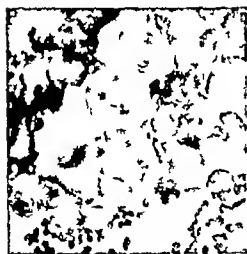


Fig 2



Fig 3

Fig 1—Lymphosarcoma. Cells indistinguishable from lymphocytes of blood

Fig 2—Carcinoma metastases to chest. Note mass of irregular cells with prominent nucleoli

Fig 3—Secondary carcinoma of pleura. Same case as Fig 2. Section of imbedded sediment showing gland acinus

Eosinophilia was seen in several fluids a point to be discussed later (Table II)

Guinea pigs injected with sediments of several ounces of fluid from cases of tuberculous pleurisy were positive in 8 cases 2 pigs died from too large a dose of sediment, and 3 were negative a much higher percentage of positive results than if small amounts had been injected as sometimes recommended

The results on abdominal fluids in Table I show much similarity to those of the chest fluids. The specific gravities were on the whole lower than in chest fluids with similar etiology, except in the cases of tuberculous where the spe

cific gravity averaged about 1.022. The transudates, whether due to cirrhosis, nephritis, heart disease, or mechanical obstruction in 3 cases of carcinoma showed low values, the majority about 1.012 or 1.013, with a few lower and some higher. The Rivalta test was negative or only faint in all the transudates and definitely positive in all the exudates, most marked in the tuberculous cases and moderate in two fluids from peritoneal carcinomatosis. The same applies to spontaneous clotting of the fluids.

Blood was found most abundant in 2 carcinomatosis cases and next in the tuberculosis fluids and was very scanty in all types of transudates, even in most of those of the cardiac decompensation group.



Fig. 4—Piece of skin found in section of pleural fluid sediment, a button cut out by aspirating needle.

The chemical findings showed high protein values in the tuberculous and carcinomatosis cases similar to those in chest cases. The lowest figures were obtained in 3 fluids from 2 nephritic cases in which only traces of albumin and globulin were seen. Cirrhosis fluids were next to the lowest, and the cardiac fluids the highest of the transudates. The albumin-globulin ratio was inconstant, although in all the tuberculous cases the globulin was greater than the albumin. Fibrin was absent or scanty in all the transudates except one, and was most abundant in the tuberculous fluids.

Nonprotein nitrogen, urea, uric acid, creatinine, chlorides, and sugar approximated values found in blood, all cases having had some blood chemistry done, either some time before or shortly after paracentesis. However, the

uric acid values in many of the cases were somewhat increased over those of the blood. A few fluids with high sugar values were found in cases of diabetes with high blood sugar.

Cholesterol content varied within all groups of cases, although there was found only a trace in three fluids from two cases of nephritis. One of these cases, from whom two fluids were drawn, had a consistently high blood cholesterol, about 350 mg per 100 cc of blood. Lecithin was not detected in the above three fluids. No constancy in regard to this substance was apparent in the other groups of cases.

The amount of inorganic phosphorus was of no differential value. Calcium determinations were fairly constant. Two nephritic fluids showed the lowest values, viz, 6.3 and 5.8 mg respectively. One fluid from a case of cirrhosis with jaundice showed a low figure, 6.8 mg, as did a fluid from a case of carcinoma totis (6.6 mg).

Cellular study of the transudates showed on the whole large numbers of classical serosal cells singly and in plaques, in most cases comprising 50 per

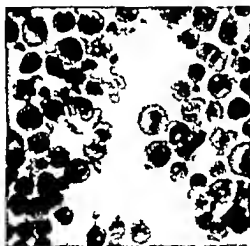


Fig 5

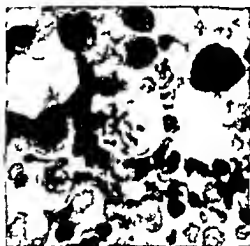


Fig 6

Fig 5—Carcinoma of peritoneum secondary to stomach cancer. Note mitotic figures.

Fig 6 Carcinoma of peritoneum secondary to ovary cancer. Note large mass of irregular cells one showing mitosis.

cent or more of the white cells seen. High lymphocytic values were seen in several cases of transudates of prolonged duration and in two tuberculous cases not previously tapped. Three fluids from carcinoma patients with fluid apparently due to mechanical obstruction showed pictures similar to those in other transudates. Cells similar to those described in tumorous fluids were found in the two fluids from cases with peritoneal metastases. Mitoses were seen in some of the cells in both fluids (Figs 5 and 6).

Guinea pig inoculations were positive in all three fluids from the tuberculous cases.

Some attention was paid to several opalescent milky abdominal fluids from nephritic and cardiac cases in an attempt to determine the chemical nature of the substance causing the chyliform appearance. In only one a cardiac case was neutral fat demonstrated. Lecithin was highest in this fluid but the values for this phospholipin in the other cases were no higher than in other fluids showing no opalescence.

TABLE II

CASE	DATE	PA TIENT	NO OF TAPS	LYMPHS	POLYS	LARGE MONOS	EOSINO PHILFS	NOTES	CLINICAL DIAGNOSIS	TYPE OF FLUID
1	3 17 28	WG	1	25	20	50	5	L monos chiefly macrophages	Postpneumonic	Pleural exudate
	3 23 28	WG	2	20	5	15	60	L monos chiefly macrophages	Postpneumonic	Pleural exudate
	4 9 27	MM	1	30	30	40	0	L monos 90% serosal, 10% macrophages	Postpneumonic pleurisy	Exudate
2	4 18 27	MM	2	30	25	30	15	L monos nearly all macrophages	Postpneumonic pleurisy	Exudate
	4 2 27	CA	1	10	75	10	5		Postpneumonic	Pleural exudate
3	7 11 27	JR	1	50	0	50	0	L monos all serosal cells	Cirrhosis of liver	Abdominal transudate
	8 20 27	JR	2	10	70	18	2	L monos partly macrophages	Cirrhosis of liver	Abdominal transudate
5	1 21 27	GM	1	50	0	50	0	L monos are serosal cells	Cirrhosis of liver	Transudate
	3 5 27	GM	2	25	7	60	8	L monos are 50% large degen erated cells	Cirrhosis of liver	Transudate
	4 11 27	GM	3	30	5	60	5	L monos are 50% large degen erated cells	Cirrhosis of liver	Transudate
6	7 2 27	GM	5	10	25	50	15	Monos as before	Postmortem sample	Transudate
	3 26 26	AV	2	23	50	25	2	L monos are 50% large degen erated forms	Tuberculous peritonitis	Abdominal exudate
7	12 22 26	FB	1	60	20	20	0	L monos chiefly large degen erated forms	Ca of lung	Pleural exudate
	1 20 27	FB	5	60	20	2	18	L monos chiefly large degen erated forms	Ca of lung	Pleural exudate
8	6 29 27	AS	4	1	93	1	5	T B Bacilli + in smear	TB pleurisy secondary	Pleural exudate
9	10 14 27	LA	5	2	20	3	75	Lung abscess	Pneumothorax (art)	Pleural exudate
10	3 27 28	WK	1	45	5	50	0	L monos all serosal cells	Cardiac decompensation	Transudate
	3 29 28	WK	2	2	60	23	15	Some macrophages seen	Cardiac decompensation	Transudate
	4 5 28	WK	3	5	10	25	60	Some macrophages seen	Cardiac decompensation	Transudate

In the other opalescent fluids the substance could not be shaken out with chloroform, ether, or alcohol. The same was true after warming with strong acid or with strong alkali. Compounds of a simple lipo protein nature therefore seem excluded. Varying the  $P_H$  considerably on both sides of the neutral point was not effective in rendering the fluid clear. No definite conclusions were reached.

Wassermann tests were done on about a quarter of the chest and abdominal fluids and the reactions were similar to those with blood serum from the same patients. Of 10 chest fluids, sent to the New York State Laboratory at Albany for tuberculosis complement fixation, 6 were reported anticomplementary, 2 from tuberculous cases were positive and 2 from nontuberculous patients were negative. Further work along this line will be continued.

In observing repeated samples from the same patient in this series, and others not included here, a definite change in the cellular picture has been noted many times, characterized by the presence of polymorphonuclear neutrophils and especially of eosinophiles (Fig 7). The presence of this latter cell following repeated pneumothorax tapplings has been repeat-



Fig 7—Post pneumonic pleurisy plus tapping. Note 60 per cent eosinophiles.

edly reported but their appearance following repeated taps in other conditions has not been described. In one case of cardiac decompensation 15 per cent eosinophiles and 60 per cent polymorphonuclears were found two days after a primary tap which showed the usual picture of a transudate with no eosinophiles and only a few polymorphonuclears. The highest percentage of eosinophiles we have ever observed is 75 per cent of the total cells, this occurring in a patient fourteen days after the first of 4 small injections of air given to compress a lung abscess. In none of these fluids have we been able to demonstrate organisms. Table II shows the counts in 10 fluids demonstrating the above phenomena.

#### SUMMARY

From the above results it is apparent that for differential diagnosis as to the type of fluid and the apparent etiology of an effusion no one method of examination can be used alone but a combination of procedures must be used. Quantitation of the various chemical substances present, except the protein content, gave no real diagnostic information and even the latter was propor-



tional to the specific gravity of the fluids. Most of the chemical substances tested for, except proteins, were found in about the same amounts as in the circulatory blood. The Rivalta test was of particular value in determining transudates from exudates but in chronic transudates with some absorption of fluid or in cases with irritation from tapping, slight positive reactions were obtained. Spontaneous clotting was most marked in inflammations, but, like the Rivalta test, was seen if transudates remained long or were concentrated by absorption in the chest, or if irritation of the serous cavity by tapping, pneumothorax, etc., was an added insult to the lining membrane. Our experiences with cytodagnosis agree fairly well with those of Sahli, namely, that the types of cells found depend on the stage of severity of the etiologic process, the duration of the effusion, secondary factors such as tapping, etc., as well as on the primary etiologic factor. Most cases of tuberculosis of the pleura, whether so called primary or secondary, show 90 per cent or more of lymphocytes, but if very acute or if tubercle bacilli are abundant enough to be found by smears, polymorphonuclears may be found in various percentages. Some chronic nontuberculous pleurisy, chronic transudates, or even fluids from malignant conditions may show nearly exclusively lymphocytes in smears. In these fluids, however, the specific gravity, Rivalta test, protein content, and above all the clinical findings, will often help to classify the fluid.

The presence of masses of large cells, irregular in size and shape, often vacuolated, showing prominent nucleoli and sometimes mitotic figures is highly suggestive of malignancy, but definite diagnosis is better made by sections of the imbedded sediment, in which fragments of tumor tissue, especially gland acini in adenocarcinoma, can often be found. Confusion results in smear examinations when degenerated forms of large mononucleated cells, either serosal desquamations or cells of other types ordinarily designated as macrophages, are seen. Absolute classification of some of these cells is certainly impossible.

The presence of large amounts of blood, if trauma in aspiration can be ruled out, suggests tumor. Tuberculous fluids as a rule show enough blood to make the small button of sediment in a 15 c c centrifuge tube appear grossly bloody. Congestive transudates as seen in hydrothorax in heart disease show practically the same amount or less than the average tuberculous fluid.

Guinea pig inoculations for tuberculosis should be made with the sediment of many ounces of the fluid examined if positive results are to be obtained. The injection of 5 c c of fluid as recommended in many clinical and laboratory textbooks is usually unsatisfactory because of the fact that the bacilli are not abundant enough in such small quantities to infect a pig.

The presence of eosinophiles in the sediment of aspirated fluids in our experience has been found to be most commonly seen in cases in which repeated aspirations have been made, occasionally appearing promptly after a single tap. These cells have been seen many times in the fluid forming after injections of air in artificial pneumothorax treatment for tuberculosis or other lung conditions. Moderate numbers have also been found in the first fluid obtained in cases of postpneumonic pleurisy, especially in patients developing postoperative lung complications. The effect of tapping in producing this

cellular picture has not been stressed in literature, and possibly some of the cases of eosinophilia in the fluids recorded heretofore under the primary etiology of the effusion may be explained on the basis of repeated tapping. We have been unable to obtain postmortem material to observe the histologic changes in the serous surfaces in any of our cases, but probably there is an aseptic inflammation present. The concomitant presence of polymorpho-nuclears or their presence without eosinophiles would help to bear out this assumption. No organisms have been demonstrated in smears or cultures in any of the fluids referred to above. The effect of novocaine injected into the pleural surface or the pleural cavity should also be considered as a possible source of the cellular picture.

#### CONCLUSIONS

After final summary of the examination of the above fluids and others not included here we have concluded that the most practical points of differential value in endeavoring to classify an effusion as to type or primary etiology are as follows:

Gross description or appearance, presence and amount of blood, presence or absence of spontaneous clotting, specific gravity, Rivalta test, microscopic study of smears of sediment, study of sections of imbedded sediment in suspected malignancy, and guinea pig injection of sediment or clot from large amounts of fluid when tuberculosis is suspected. In very few fluids will only one of the above methods give conclusive evidence, but if the clinical features of the case with the history of the duration of the effusion, information as to previous aspirations, etc., are known, a fairly reliable diagnosis can be made if the various methods just mentioned are used. Chemical examination for substances other than protein is of no real diagnostic value. Possibly complement fixation tests for tuberculosis may be worth consideration a point now under investigation by us.

#### REFERENCES

- 1 Wells, H. G. *Chemical Pathology* ed 4 Philadelphia W. B. Saunders Co., p 790 1925
- 2 Gorecki, Z. *Recherches Chimiques sur les liquides séreux*, *Rev de Méd* 41 470-484, 1924
- 3 Bezancon, F., Weil, M. P. and Guillaumin, C. O. *La Constitution Physico-Chimique des Liquides Pleuraux*, *Ann de méd* 21 316-330 1927
- 4 Bernhard, A. *The Chemistry of Transudates and Exudates With Reference to Their Nonprotein Nitrogenous Constituents*, *Interstate M. J.* 25 188-191, 1918
- 5 Nathan, H. *Ueber den Zuckergehalt Pathologischer Flüssigkeiten*, *Klin Wchnschr*, 6 1516-17, 1927
- 6 Orsi, A. *Il Contenuto in Glucosio dei Liquidi Patologici delle Grandi Sierose ed il suo Valore Diagnostico*, *Pathologica* 18 482-484 1926
- 7 Sahli, Hermann. *A Treatise on Diagnostic Methods of Examinations* Philadelphia W. B. Saunders Co., p 1229 1914

- 8 Bock, E Zur Mikroskopischen Diagnose von Geschwülsten der Pleura aus Punktionsflüssigkeit, Klin Wehnschr 4 651 652, 1925
- 9 Zemansky, A P The Examination of Fluids for Tumor Cells, Am J M Sc, 175 489 504, 1928
- 10 Miller, J L Transudates and Exudates, With Report on 75 Fluids Am Med 8 835 843, 1904
- 11 Unverricht Die Klinisch Wichtigsten Methoden der Prüfung von Pleura und Ascitesflüssigkeit, Klin Wehnschr 5 2029 2030, 1926
- 12 Simon, C E A Manual of Clinical Diagnosis by Means of Laboratory Methods for Students, Hospital Physicians and Practitioners, ed 9, Philadelphia, Lea & Febiger, p 851, 1922
- 13 Machailow, K Complement Fixation and Bacteriology of Exudates, Ztschr f Tuberk 43 191, 1925
- 14 Salomon, M, and Valtis, J Recherche Comparative des Anticorps et de l'antigène Tuberculeux dans le sang et les Liquides Pleuraux, Compt rend Soc de Biol 93 1145 1146, 1925
- 15 Pousini, G Sulla Reazione di Wassermann nei Liquidi da Versamento, Riforma Med 42 51 54, 1926

## DISCUSSION

*Dr Frederic E Sondern*—I would like to ask Dr Foord if he will tell us about the abdominal fluid from cases numbers 16 to 20, whether chyle was actually demonstrated, and if not whether he was able to determine the cause of the turbidity

*Dr Kano Ikeda*—Some years ago in our laboratory we studied the chemistry of body fluids in comparison with blood chemistry. We found the same kind of results as Dr Foord. The thing I would like to suggest here is this. We do a postmortem examination on a patient who has been in the hospital a very short time or whose diagnosis has been very obscure. On these cases it has been my custom to examine the pericardial fluid chemically. I have found at least one determination which is of value in arriving at a cause of death, namely, the determination of the fluid for urea nitrogen. Sometimes we have great difficulty in determining whether or not the patient had died of uremia. The urea nitrogen will show a marked increase in the pericardial fluid in uremic death. As far as the other constituents are concerned, I am not prepared to say. Sugar of course decreases very rapidly. If the sugar content remains, say 0.20 per cent, that gives us a clue that the patient may have had diabetes. I think it is a good practice for pathologists performing postmortems to do chemical analyses on the pericardial fluid. We find the same thing holds true with other body fluids.

*Dr Alvin G Foord* (closing)—In answer to Dr Sondern I must say that probably I should have called the opalescent fluids "pseudo chylous" rather than "chyliform," because of the difficulties in demonstrating fat content. Dr Youngberg, as well as other chemists, has been able to learn very little about the cause of the milkiness of these fluids, and also in reading reviews on the subject, particularly as given in Well's Chemical Pathology, there is found considerable disagreement on the subject.

In answer to the warning as to the diagnosis of tumor on single cells, I can say that I quite agree. At least I am not able to make such a diagnosis on tissues or serous fluids, although it appears to me that the latter would afford splendid opportunities for single cell study. The best way to handle sediments examined for tumor cells is by making sections of the imbedded sediment and searching the same for tumor particles, such as acini, etc. Once again I wish to reiterate that conclusions on fluids are best made after clinical study of the patient, and with a fair impression of the clinical problem involved, the laboratory evidence will be of more value.

*Dr A H Sanford*—We get a chocolate colored fluid with endothelial cells in plaques in it and we guess that it is a case of malignancy. We have had men who have worked with Dr MacCarty who know all about single cell diagnosis, examine such fluids. I never could get any of them to find any cells that they would call malignant. All of us have seen fluids that we think are malignant. We have not time to go into that but I think this is something important.

## PRIMARY CARCINOMA OF THE FALLOPIAN TUBES\*†

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THE first description of carcinoma of the fallopian tubes was given by Orthmann in 1886 Just forty years later, Wechsler<sup>1</sup> found 192 tubal carcinomas in the literature and added four additional cases Since this article we have found seven other instances in the literature Cameron<sup>2</sup> reported two, Ewing,<sup>3</sup> one, Beck,<sup>4</sup> one, Liang,<sup>5</sup> one, Heil,<sup>6</sup> one, Bower and Clark,<sup>7</sup> one, and we are adding two additional cases occurring in the surgical service of the White Cross Hospital and Ohio State University, bringing the total number of recorded cases to 201

Therefore, it becomes evident that primary carcinoma of the fallopian tubes is a rather rare condition and that in comparison with the total pathologic lesions of this organ the percentage of malignancies is very small indeed Of 19,000 gynecologic cases in Johns Hopkins Hospital (Vest<sup>8</sup>) only four instances of primary cancer were observed Wechsler reported four cases out of 5,870 specimens at Lenox Hill Hospital, while the present two cases have occurred in a series of 5,000 surgical specimens As the condition is often recognized only histologically, there are no doubt cases which have been overlooked where routine microscopic examinations were not made

The literature of the subject has been so thoroughly covered by Wechsler in his recent article, that it is not the purpose of this paper to go into a complete comparative study but to record only those cases that have occurred since his report and to add those occurring in our laboratories Two of these cases have been bilateral, two have occurred in the right tube and three in the left

CASE 1—The tissues under consideration were referred by Doctor Joseph Price of Mercy Hospital, with the diagnosis of an "old inflammatory process of the uterus, left ovary and tube" By his permission we are able to report this case

Mrs K, aged forty seven years, was admitted to Mercy Hospital, April 4, 1927, with a complaint of pain in the pelvis

History—The patient has had only the ordinary diseases of childhood but has always been in delicate health There have been no operations and no history of venereal disease Pneumonia at the age of sixteen years with complete recovery At seventeen to eighteen years she had night sweats for two months Menstruation started at the age of fourteen years of the twenty three to twenty four day type often irregular but otherwise normal There has been one normal delivery

The present illness started with pain in the pelvic region in November 1926 Treatment afforded no relief About March 1, 1927, the pain became more severe and radiated into the groin and thigh, though limited to the left side She complained of nocturia and

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painful defecation, but her stools were free from blood. There was no vaginal discharge. Laboratory findings revealed a moderate anemia, otherwise nothing noteworthy. The preoperative diagnosis was multiple fibroids and an inflammatory mass on the left side of the pelvis. Panhysterectomy and appendectomy were done.

*Macroscopic Description*—The specimen consisted of the uterus, right tube and ovary, which were sealed into a solid mass measuring 4.5 by 5 cm. No definite relationship of the tube and ovary could be made out, the tube was markedly distended, resembling an old pyosalpinx. On section the lumen of the tube was dilated, filled with a thick, yellow debris, and lined with short papillary outgrowths which showed numerous small areas of necrosis. The process did not suggest itself even at this time as being of a malignant nature.

The uterus was small, very firm in consistency. The endometrium was slightly thickened, measuring 4 mm. There were no tumor masses in the uterine wall or in the endometrium.

The opposite tube was normal in size, the fimbriated end was not sealed. The ovary was small with many scars over its surface.

*Microscopic Findings*—Sections of the large mass show the tissue to be edematous and infiltrated with leucocytes, especially in small areas which simulated abscess formation. In addition to this inflammatory process the epithelium, which resembled the tubal mucosa, was decidedly hyperplastic, atypical in its method of growth and presented mitotic figures. There was a very definite infiltration of the muscular wall by masses of epithelial cells.

Sections from the uterus and cervix showed no invasion by the malignant growth.

#### *Diagnosis*—

- 1 Primary papillary alveolar carcinoma of the left uterine tube

CASE 2—(A somewhat more typical history) Mrs. L. S. S., aged sixty-one years, was admitted to White Cross Hospital on February 9, 1928, complaining of a bloody vaginal discharge and pain in the back.

The menses began at the age of thirteen years, were regular and normal. At the age of seventeen she became pregnant and aborted at three months, with normal recovery. There is no history of venereal disease. The menopause occurred at the age of forty-eight years when menstruation stopped completely. There was no further trouble until twelve years later, when she noticed a watery vaginal discharge which was sometimes bloody and at times profuse. She entered a hospital and a curettage of the uterus was performed. The curettments were found to be negative and she was assured at this time that she would entirely recover. However, since that time, she has complained of constant discharge which upon numerous occasions has become bloody. She has also complained of continual pain in her back during this period. There has been no loss of weight and otherwise the patient has been in apparently normal health.

The first of February, 1928, the discharge had become more profuse. On pelvic examination a slight tenderness could be elicited over the region of the left tube, though no definite diagnosis could be made.

On February 9, she entered the hospital at which time the Wassermann and Kahn tests were negative. Her pulse was 80, temperature 98, blood count, blood chemistry, and urinalysis were not unusual, other than a slight leucopenia. Her appetite was good and she complained of no constipation. She has urinated twice a night during the last few months.

An exploratory operation was advised with a preoperative diagnosis of left tubo-ovarian infection or uterine neoplasm.

*Operative Notes*—The uterus and cervix were small and atrophic. There was a large amount of periuterine adhesions. The right tube and ovary were apparently normal. The left tube was markedly enlarged, soft and cyst-like, and the fimbriated end sealed.

A left salpingo-oophorectomy and a right salpingectomy were performed.

*Macroscopic Findings*—The specimen consisted of the right and left fallopian tubes and a portion of the left ovary. The ovary measured 2 cm. by 1½ cm. by 1 cm., and was somewhat atrophic. On section there was revealed three or four small follicular cysts. There

was no evidence of tumor nodules. Upon opening the left tube a large papillomatous outgrowth was revealed covering the entire inner surface of the tubal wall and everting its edges. The tube measured 8 cm by 6 cm by 6 cm. The tumor mass consisted of multiple long finger like projections of the tubal mucosa, greyish white in color, arborescent and very friable. There was a small amount of serous exudate within the lumen and occasional dark areas of hemorrhage. Necrosis was not marked. The tubal musculature was thickened and firm. The peritoneal coat presented a few fibrous adhesions.

The opposite tube measures  $5\frac{1}{2}$  cm in length by  $1\frac{1}{4}$  cm in diameter and was kinked and tortuous. On section the wall was generally thickened by fibrous tissue and the mucous membrane was absent.

*Microscopic Findings*—Section of the left fallopian tube, throughout its enlarged portion, revealed numerous papillary outgrowths of the tubal folds, consisting of a central stroma covered with cuboidal and polyhedral epithelium which occurred in a single or multiple layers and in some areas in the form of solid cellular strands. Mitosis was abundant. Imbedded deeply in the musculature of the tube were masses of epithelial cells some in alveolar arrangement some in solid masses in all regards similar to the picture seen in gastric carcinoma. The serous coat in no instance was invaded. There was a marked round cell infiltration of the muscle wall.

The right tube showed marked fibrosis of the muscularis with obliteration of the mucous folds.

The ovary revealed no invasion of malignant cells.

#### *Diagnosis*—

- 1 Papillary carcinoma of the left fallopian tube
- 2 Right chronic salpingitis

*Note*—This patient returned to the hospital eleven months following removal of the carcinomatous tube presenting three nodular masses in the abdominal scar, measuring 2 cm in diameter. The lower abdomen was painful and tender. Upon opening the abdomen the omentum was found to consist of a mass of carcinomatous nodules closely attached to the transverse colon. Both the visceral and parietal layers of the peritoneum were extensively studded with similar nodules. The uterus itself was small and free from invasions. The metastatic nodules were limited to the lower portion of the abdomen and mesenteric nodes.

*Pathology*—Macroscopic examination revealed numerous soft whitish tumor masses which were papillomatous and friable. The microscope presented the picture of adenocarcinoma resembling in close detail that described as occurring in the fallopian tube eleven months previously. The case was inoperable and the patient left the hospital with a hopeless prognosis.

The most frequent and pronounced symptoms of carcinoma of the fallopian tube are, a profuse serous, often bloody or serosanguinous, vaginal discharge, pain, irregularity of the menses in younger women and negative curettements. Tumor masses may or may not be palpated. Constipation, nocturia, anemia and weakness are frequent associations in the latter part of the disease.

Both of the cases here reported fell in the postclimacteric period. One case presented a persistent serous and spasmodically bloody discharge of a year's duration, pain in the back and tenderness in the left lower quadrant of the abdomen, while the other was entirely free from any discharge but in neither of these cases, was the malignant nature of the disease suspected before operation.

## SUMMARY

1 In this paper we have reported seven additional cases of primary carcinoma of the fallopian tube in the literature and two occurring in our own laboratory, bringing the total number reported to 201

2 The most common clinical symptoms of the disease are those of vaginal discharge, often bloody, pain in the pelvis, tumor mass, and, late in the disease, constipation and nocturia

3 The gross appearance of tubal carcinoma is often deceiving and its presence may be entirely overlooked unless careful routine microscopic examinations are made

4 The microscopic picture is usually that of papillary-alveolar carcinoma

## REFERENCES

- 1 Wechsler, H F Primary Carcinoma of the Fallopian Tubes, Arch Path & Lab Med 2 161 205, 1926
- 2 Cameron, S J Malignant Disease of the Ovaries and Fallopian Tubes, Brit M J 2 285 288, 1925
- 3 Ewing, J Neoplastic Diseases, ed 3, Philadelphia, W B Saunders Company, 1127 1928
- 4 Beck, Walter Primares Tubenkarzinom, Zentralbl f Gynak 50 1503 1505, 1926
- 5 Liang, Z Was lehrt das primäre Tubencarcinomen pathologischer Hinsicht? Virchow's Arch f path Anat 259 576 607, 1926
- 6 Heil, Karl Primares Tubenkarzinom, Zentralbl f Gynak 50 2952 2958, 1926
- 7 Bower, J O, and Clark, J H Primary Bilateral Carcinoma of the Fallopian Tubes, Arch Surg 11 586 597, 1925
- 8 Vest, Cecil W A Clinical Study of Primary Carcinoma of the Fallopian Tubes, Bull Johns Hopkins Hosp 25 305 317, 1914

## DISCUSSION

*Dr A S Giordano*—I had one case like that I had never seen one before and was very doubtful about saying what it was

*Dr Clarence I Owen*—I would like to ask about recurrence and metastasis

*Dr Ernest Scott* (closing)—One of the cases died a few days after operation and the other was operated upon only sixty days ago

# THE INTERPRETATION OF BORDERLINE ALLERGIC REACTIONS\*

BY WARREN T. VAUGHAN, M.D., RICHMOND, VA

THE interpretation of allergic skin reactions has passed through a variety of evolutionary phases. Even now the procedure has recognized shortcomings.

## THE DELAYED REACTION

At the beginning the immediate or half hour reaction was alone recorded and no reaction was considered positive by the scratch method unless accompanied by an urticarial wheal preferably with pseudopod formation and surrounding erythema. In 1922 and 1923 Peshkin and Rost,<sup>1</sup> Sidlick and Knowles,<sup>2</sup> Shannon,<sup>3</sup> Vaughan,<sup>4</sup> and others called attention to delayed types of the reaction usually showing up after an interval of about twenty four hours.

Vaughan suggested that the mild immediate reaction and the delayed positive reaction constituted the logical type of response in those cases in which the protein poisoning was chronic as with foods eaten daily, and that these reactions were the clinical counterpart of the well known experimental Arthus phenomenon. The latter represents chronic protein poisoning following daily local injections of horse serum, manifesting itself as a soft infiltration which later becomes hard, edematous and even necrotic.

Most allergists I believe now make the twenty four hour reading along with the half hour study. A good positive delayed reaction is never as pronounced as the strongly positive immediate reaction never progressing to the formation of an urticarial wheal but it may be much more strongly positive than was the immediate reaction to the same protein. Indeed a good positive delayed reaction may follow an entirely negative immediate reaction. The delayed reaction consists of slight induration along the line of scratch with surrounding erythema usually rather sharply demarcated, of from one to five centimeters diameter. It looks rather like a beginning infection but fails to progress as such.

Not infrequently persons known through experience to be sensitive to certain proteins display persistently negative skin reactions to these same proteins. Many such cases reported negative to the immediate reaction would have shown positive delayed reactions. The man who makes only half hourly readings will miss the not infrequent delayed positive reaction, a reaction which I consider equally as specific as the immediate one, and will erroneously conclude that the reaction to the protein was entirely negative.

The selection of twenty four hours as the time interval for the reading of the delayed reaction is purely arbitrary. The reaction is usually pretty well faded by the end of forty eight hours but in rare instances it will persist two or even three days. It is not necessarily at its maximum after twenty four

\*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 6, 9 and 11, 1925.



hours. It may already be fading. I have sometimes obtained negative or nearly negative half-hour and twenty-four-hour reactions with a clearly positive response at the end of four to six hours. I therefore make it a routine procedure to take three readings, the first at the end of twenty to thirty minutes, the second after from four to six hours and the third on the following day. With this routine one is very likely to catch the delayed positive reaction at some stage in its evolution. Even then the maximum reaction may have been missed. I have seen cases in which the maximum response occurred at the end of two hours but the four- to six-hour reading was still sufficiently strong so that the value of the reading was not lost.

#### BORDERLINE REACTIONS

The man who insists upon a clear-cut positive reaction and fails to consider the borderline response will often miss the specific allergenic protein. The response is not necessarily maximal. The scratch method is rather a crude procedure but when carefully carried out is surprisingly accurate as checked by repetitions of the test. But we cannot be certain that enough protein has passed into solution or enough has actually come into contact with the tissue cells to provoke a maximum response. Perhaps there was just enough to give only a mild reaction. I have often seen the drop of dissolved protein spread over an area of half an inch or more surrounding the scratch, the manipulator evidently failing to realize that only along the line of the abrasion is one capable of eliciting any response.

We know that immediately following an acute exacerbation negative responses are often obtained to proteins which at other times give positive reactions in the same individual. I do not imagine that a patient in such a negative phase changes suddenly from being a negative reactor to a strongly positive one. There are undoubtedly gradations during which the reaction may be borderline.

#### GRADES OF REACTION

The immediate reaction is the one of greatest value but the early and late delayed reactions give sufficient information so that we cannot afford to overlook them. An immediate reaction with a definite urticarial wheal and usually some surrounding erythema I class as one-plus. More strongly positive reactions are two-plus, three-plus, etc. The borderline immediate reaction with no wheal but with more erythema than those of its neighbors is plus minus. I even designate a third group, those in which the reaction can scarcely even be called questionable as plus minus minus. These are just sufficiently different from their neighbors to be termed barely suggestive. The one-plus delayed reaction shows an erythema of say one or one and one-half centimeters in diameter. Above this we observe two-plus and three-plus reactions and below it as on the immediate, questionable or plus minus and barely suggestive or plus minus minus reactions.

An isolated plus minus minus reaction receives no further consideration. But if on all three readings which have been made without reference to each other a particular protein shows up as plus minus minus each of the three times, the protein may be one of etiologic significance and is so considered.

In the same way a plus minus reaction on two or three of the three readings is given consideration and an experimental trial

In this way I believe that I have increased my efficiency in detecting etiologic proteins. Many of the proteins incriminated in this manner are later found not to be factors in the individual case but others which have given only borderline reactions have sometimes been shown unequivocally responsible for symptoms

#### ANALYSIS OF READINGS IN A SERIES OF BENEFITED CASES

A small series of 39 eczema cases all relieved by protein avoidance only was analyzed as to the character of the reactions. Clearly positive immediate reactions occurred in 61.5 per cent. Thirty five per cent presented clearly positive delayed reactions. Often the immediate and delayed reactions occurred in the same individual but in 17.9 per cent the delayed reaction was relatively more intense than the immediate. In 17.9 per cent the diagnosis was based entirely upon delayed reactions the immediate being no stronger than plus minus. Of these latter cases four showed the strongest reaction at the end of from four to six hours and three at the end of twenty four hours

#### RESULTS OF TREATMENT IN BORDERLINE AND DELAYED REACTORS

In 1926<sup>5</sup> I reviewed a series of 135 allergies comprising groups of respiratory and cutaneous allergy (Table I). One hundred and thirty two had had the complete routine skin test with three readings. Of these, 46 per cent showed a clearly positive immediate reaction, 7 per cent a clearly positive delayed reaction and 47 per cent gave at best only borderline reactions on all three readings (Table II).

TABLE I  
ALLERGIC DISEASES STUDIED

DISEASE	NO CASES	NO BENEFITED	PER CENT BENEFITED
Asthma	48	52	68
Eczema	28	15	54
Pruritus ani	22	9	41
Vasomotor rhinitis	15	11	73
Urticaria	22	16	73
Total	135		61.5

TABLE II  
DISTRIBUTION OF CASES ACCORDING TO CHARACTER OF ORIGINAL SKIN TEST

	CASES	PER CENT
Good Prompt Reaction	61	46
Good Delayed Reaction	9	7
Borderline Reaction	62	47
Total	132	100

The question naturally arises as to which of these three groups experienced greatest benefit: those with good prompt reactions, those with good delayed reactions, or those with poor reactions. Table III answers this question. Table IV is but a condensation of Table III in which the results are grouped and may be more readily read. In the third column in Table III those receiv-

ing but 50 per cent improvement are included since I feel that with no other treatment prescribed these individuals may attribute such improvement as they have had directly to protein avoidance even though the results in the 50 per cent group have not been sufficiently good so that the patient was satisfied

TABLE III  
PROGNOSTIC VALUE OF SKIN TESTS  
COMPARISON OF ORIGINAL SKIN TESTS WITH ULTIMATE RESULTS

EXTENT OF IMPROVEMENT	COMPLETE RELIEF				
	NONE (0%)	SLIGHT (25%)	DISTINCT (50%)	VERY GOOD (75%)	RELIEF (100%)
Good Prompt Reaction (61 cases)	18	15	13	36	18
Good Delayed Reaction (9 cases)	22	11	11		56
Borderline Reaction (62 cases)	37	13	16	19	15
Total, 132 cases	27	14	14	26	19

TABLE IV  
PROGNOSTIC VALUE OF SKIN TESTS  
(Continued)

EXTENT OF IMPROVEMENT	UNSATISFACTORY	VERY SATISFACTORY	DIRECT BENEFIT
	(0-25%)	(75-100%)	(50, 75-100%)
Good Prompt Reaction	33	54	67
Good Delayed Reaction	33	56	67
Borderline Reaction	50	34	50
Total	41	40	19

TABLE V  
DISTRIBUTION OF REACTIONS ACCORDING TO END RESULTS

NO	PROMPT REACTION	DELAYED REACTION	GOOD REACTION	POOR REACTION
	PER CENT	PER CENT	PER CENT	PER CENT
36 Unimproved	31	5	36	64
54 Little benefit	37	55	47.5	58.5
59 Very satisfactory	56	8	64	36
25 Entire relief	44	20	64	36

Referring for the present purpose only to the first two columns we see that with good prompt reactions and good delayed reactions the percentage of satisfactory results is decidedly higher than that of unsatisfactory results. Indeed, for every patient not benefited there were two who experienced appreciable measures of relief directly attributable to the protein avoidance.

But the significant fact brought out in this table is in the last group, those showing only borderline reactions. Thirty-four per cent of these obtained very satisfactory results from protein avoidance only. For every borderline reactor unimproved there was another which showed evidence of direct benefit. This would appear to substantiate my claim that in many clinics not enough attention is being paid to the borderline reactions.

#### ANALYSIS OF UNIMPROVED CASES

Table V brings out a new observation. Of those cases who received no benefit whatsoever 36 per cent had given a good clear-cut positive sensitization reaction. Why did these good reactors show no improvement following specific protein avoidance?

In 1927<sup>6</sup> I made a study of 71 consecutive cases of eczema. Thirty nine or 55 per cent were relieved of the eczema by protein avoidance only. Forty five per cent were not sufficiently relieved to consider the procedure satisfactory. Further study of these two groups, the *good result* and the *poor result* series, brings out some interesting facts.

Clearly positive prompt reactions were obtained in 61.5 per cent of the 39 good result cases. But they were obtained in almost as high proportion in the poor result cases, namely, 50 per cent.

Complete past and family histories were obtained in 39 of the entire series, 19 being good result cases and 20 poor results. Eight of the former gave a positive past history for one or more other allergic diseases. But eight of the latter gave equally definite positive histories.

Eleven of eighteen good result cases gave a positive family allergic history. Against this fourteen of the nineteen poor result cases in which family history was obtained gave a positive family history for allergy.

Seven of eighteen good result cases gave a family history of eczema and six of nineteen among the poor results did likewise.

A clear cut delayed positive reaction was found in seventeen of thirty nine good result cases and seventeen of thirty two with poor results.

Although one series obtained satisfactory relief from protein avoidance and the other little or none, the evidence just recorded suggests strongly that allergy is a factor even in those cases which were not relieved.

#### TECHNIC

A word with regard to the method of making the test. The arm and forearm are still used for scratch or intradermal testing by the majority of allergists. I have discarded this site some time ago in favor of the back. The individual nerve terminations are more widely separated in the skin of the back than elsewhere and the back is therefore less sensitive to pain. The patient does not see the scratching or needling and the psychic pain factor is thus to a great extent eliminated. This is especially helpful in children. I have compared the strength of the reaction in a small series of known reactors, on the back, the arm, the forearm, and the thigh and have found no great variation in the intensity of reaction in these areas. Six cases were studied in all. The reaction on the back was as large as or larger than elsewhere in every case except one. Even in this latter it was clearly positive. The comparison was rather rough since the scratch method was used but this method was chosen since it is precisely that which is used in the actual work of testing.

Alexander<sup>7</sup> has studied the reactivity of the skin to test proteins at various sites of the body with carefully controlled experiments using intradermal inoculations of very carefully measured amounts and always in duplicate. In such places as the forearm, the back, abdomen, the leg and the thigh he finds varying cutaneous response or sensitiveness depending upon the location even when the tests are practically simultaneously applied. The skin of the back and of the abdomen usually gave a slightly stronger reaction than the other areas mentioned although occasionally the reverse was the case.

In experimental work with histamine, using dogs, he found some variation in sensitivity of the skin in different areas

Any number of tests may be applied on the back at one sitting, one hundred being easily given with no discomfort to the patient and no crowding of the scratches. The esthetic advantage of scratches in a location where they will not show are appreciated especially by the feminine allergies.

There is no more justification for applying the temporarily disfiguring skin tests to the arms than there is for producing a great smallpox scar in a locality that is often exposed. Doctors are great creatures of habit.

Fig 1 illustrates the method of the dorsal application and brings out how much easier it is to apply the scratches or the intradermal test to one continuous surface rather than to upper and lower circumferences of two arms. In the case of men the factor of extensive distribution of hair does not so often require circumvention.



Fig 1—Illustrating method of applying sensitization tests to the back at one sitting

Most writers recommend a one-eighth inch scratch for the application of the protein. Such a short scratch may be esthetic but it lessens the probability of sufficient protein coming in contact with the tissue cells to produce reaction. My scratches are about three-eighths of an inch long. I find that with these larger scratches the delayed reaction particularly is more easily read. I have yet to see a case of infection from the application of the protein test either by scratch or intradermally.

In the event of borderline reactions, we are left two alternatives, therapeutic trial by elimination and subsequent exposure, or the intradermal test. The intradermal reaction is often positive when the scratch has been negative, particularly in adults. This latter alternative should be the method of choice especially in those cases where specific desensitization is contemplated. Where there is reason to suspect a given protein, even the intradermal reaction should not be considered absolutely negative until after the application of a highly concentrated test extract.

Occasionally the reactions to scratch and to intradermal inoculations are so mild and yet the history is so definite that subcutaneous and ophthalmic

reactions become necessary. Many writers have mentioned the subcutaneous method, the more recent writers being Kahn and Grotbaas<sup>8</sup> and Balyeat.<sup>9</sup> The former authors find that in Texas where pollen prevalence is extremely high through most of the year, persons with such mild sensitiveness will not react by scratch or intradermally, nevertheless they are specifically sensitive to pollens, a fact which can be demonstrated by the subcutaneous reaction.

### CONCLUSIONS

There are many factors to be considered in the performance and interpretation of sensitization tests but I would emphasize that if two delayed readings are made in every case, pollens excepted, if more attention be paid to the borderline reaction especially when it occurs on two or more of the three readings, if the scratch be made the proper length and if questionable scratch reactions are checked by intradermal tests, there will be fewer false negative reports, there will be fewer occasions for repeating the entire series, and therapeutic efficiency will be increased.

### REFERENCES

- 1 Peshkin, and Rost. *Am J Dis Child* 23 51 1922
- 2 Sidlick, and Knowles. *Am J Dis Child* 23 316, 1922
- 3 Shannon. *Ibid* p 392
- 4 Vaughan, W T. *South M J* 17 749 October, 1924
- 5 Vaughan, W T. *South Med & Surg* July 1926
- 6 Vaughan, W T. *J Lab & Clin Med* 13 24 Oct, 1927
- 7 Alexander Henry L. Communication Before the Association for the Study of Asthma and Allied Conditions. Washington, D C, May 28, 1928
- 8 Kahn I S, and Grotbaas Emma M. *M J & Rec* March 3 1926
- 9 Balyeat, Ray M. *J Oklahoma M A* August 1926

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### DISCUSSION

*Dr J H Black*—Dr Vaughan's paper I think is of value because it brings up two points which are very important. First it proves very definitely that a lot of literature that is being sent out is quite fallacious. Literature is sent out giving the idea that the tests are perfectly easy to interpret. I think this type of paper illustrates very definitely that this is not true. Second men doing this type of work are doing it in as many different ways as we are doing Wassermanns. Some are doing intradermal and some cutaneous tests. There is no uniformity as to the method in use. There is also a great deal of difference in the method of reporting results.

The interesting thing in this work is the possible relationship of his delayed reaction to the absence of "allergic antibody" in the blood. We have found that certain individuals who were entirely negative to intradermal injection showed no antibody in the blood. We have believed that the negative reaction depended upon the absence of antibody. I should like to ask if Dr Vaughan has made any effort to determine whether this is the case with those showing delayed reaction and also whether he finds delayed reactions in patients giving a negative intradermal reaction.

*Dr Wm G Exton*—I would like to ask Dr Vaughan as an allergist for some explanation of the wheals we occasionally see immediately after inserting the needle into the veins of the arm in order to get blood specimens. They have interested me for a number of years. Do cleaning fluids have anything to do with it or is it just a reaction from the needle prick? Or what other explanation would an allergist give?

*Dr A H Sanford*—I should like to ask Dr Vaughan to discuss something that may be what Dr Exton refers to that is what his procedure is in the hypersensitive skin especially with intradermal tests where you get a reaction to everything including the control.

*Dr Warren T Vaughan* (closing) —With regard to Dr Exton's question, at the bend of the elbow you get more reaction than either above or below, and we avoid the bend of the elbow I feel that most of this is purely traumatic. The tissue in the bend of the elbow is much looser than the skin above and below, and there is more room for swelling. If you get too near the elbow you may get false positives.

I have not, Dr Black, made any special studies along that line. There was an interesting paper presented at the Washington meeting of the Asthma Society which might bear short repetition. It came from Philadelphia, bearing on the use of immune serum. A positive skin reaction to horse serum does not necessarily mean that the patient will get asthma after serum injection because the reactivity might be in the skin alone. When a positive skin reaction was obtained on the patient, the technic was to take the blood from the patient, inoculate it intradermally into another individual, and make a skin test at the site of intradermal inoculation. If you again get a positive reaction the "reagents" are also in the patient's blood. Then, of course, the patient may get asthma as well as the hives, following serum treatment.

We do see delayed positive intradermal reactions, which are sometimes more clearly positive than the immediate reaction to the same protein.

The nonspecific reaction is a problem. I do not know of any solution as yet. You will notice that in the picture of the back there were a lot of reactions. Sometimes where you have general cutaneous irritability there is enough variation in the degree of reaction to give you a lead and you can try out therapy on the basis of relative size of reactions. Sometimes the delayed reaction will help a great deal in these general reactors. There is less tendency for nonspecific generalized delayed skin reactions. Occasionally a series of peptone injections will lessen the nonspecific tendency so that later skin testing will bring out only the truly positive substances.

# A METHOD FOR MEASURING THE BACTERICIDAL ACTION OF WHOLE BLOOD AGAINST GRAM POSITIVE COCCI\*†

BY WILLIAM THALHIMER, M D, AND CHARLOTTE COLWELL, A B,  
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THIS report deals with the development of a method for accurately determining the ability of whole blood to kill gram positive cocci in vitro, particularly *Staphylococcus aureus*. When this investigation was started, a simple and accurate method of this sort was not available. An accurate bactericidal method is needed, since many investigators have found that the presence in blood of antihodies, such as agglutinins, precipitins, complement fixing bodies, and others, does not indicate the degree of bactericidal power of the blood. It is evident that many clinical problems and problems in immunity which are in need of investigation can be studied with the aid of a proper method.

The problem of determining the bactericidal power of blood, or even of serum, against gram negative organisms, such as *B. typhosus*, is a comparatively simple one, and methods for this have been available for a long time. The method of Denison<sup>1</sup> is excellent, and soon after it was described one of us (W T) used it in an attempt to determine the bactericidal power of blood and serum against gram positive cocci. In these unpublished experiments, whereas the results of Denison were corroborated, no killing effect was found on gram positive cocci. Whole blood kept from clotting by potassium oxalate and small amounts of hirudin, and serum, with and without the addition of guinea pig serum, were studied. In the light of the experience gained in the present study, the most probable explanation of the former negative results is that the blood was diluted and was not allowed a sufficient period of time to act on the bacteria before the mixtures were plated.

The failure of Denison's method with gram positive cocci should not be considered in any way as a reflection on this method when used for gram negative bacilli. Denison's method does not seem to have gained the prominence which it deserves.

About two years ago, at the request of Dr C H Davis, a study of the bactericidal power of blood was made, using the method described by Heist and his coworkers. In this method five capillary tubes are used, into which are introduced the same amount of different suspensions of pneumococci. Each of these suspensions is a 1:5 dilution of the previous one. The fluid is then drawn or blown out of each tube, leaving some pneumococci deposited on the inside of the wall. Immediately after this each tube is filled with whole unclotted blood to the same mark used to measure the pneumococcus suspension. The end of the tube is sealed with paraffin, and the tubes are incubated for twenty

From the Laboratories of Columbia Hospital Milwaukee Wisconsin.

\*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis Minnesota, June 8, 9 and 11, 1918.



four hours At the end of this time the blood-bacterial mixtures are blown out on a series of slides, dried, and stained for bacteria, so as to determine whether or not the bacteria have increased or decreased in number, and the degree to which this has occurred We found, as did Matsunami and Kolmer,<sup>3</sup> that one can detect gross differences in the bactericidal activity of whole blood by this method We found, as did these workers, that we could not detect slight differences in bactericidal power, nor did the results in duplicate tests check sufficiently close to one another to establish confidence in the method

We then abandoned this method and did not resume our studies until December, 1926, at the request of J L Yates At this time the chief object was to devise a bactericidal test that could be used for determining the best time for using donors in performing immuno-transfusions\* At this time, although we applied the principles of Heist's capillary tube method, we worked with amounts of whole blood and bacterial suspension large enough to be measured accurately with pipettes

The first problem was to secure whole sterile blood that would not clot This was easily solved by using small amounts of the anticoagulant heparin, as this substance can be sterilized readily by boiling or autoclaving Other reasons for the choice of heparin were that it was inconceivable that such small amounts as were necessary to keep blood from clotting could alter in any way the bactericidal power of the blood, and because other substances, such as potassium citrate or oxalate, are believed by Colebrook and Storei<sup>4</sup> to decrease the bactericidal power of blood

Following the scheme of Heist, our first tests were made using different samples of the same blood seeded with different numbers of *Staphylococcus aureus* This method Sir A E Wright<sup>5</sup> has called "the implanting and explanting procedure," in contradistinction to the implanting and inculturing procedure

A measured amount, 0.5 c.c., of each blood-bacterial mixture was inoculated immediately into a tube of melted agar, plated, incubated, and the colonies counted This gave the essential control The blood-bacterial mixtures were put in the thermostat and 0.5 c.c. was removed at two-, four-, six-, and eight-hour intervals, and plated in the same manner as the control The flask containing the blood-bacterial mixture was thoroughly shaken immediately before each sample was removed Our early tests showed a marked diminution of living organisms at the end of four hours' incubation For a while we arbitrarily used this interval in the tests, but continued to use several samples of blood, each with a different number of bacteria (See Table I)

The first test yielded a very unexpected finding Although there were 5 times as many bacteria in the third set-up as in the fourth, and 20 times as many as in the fifth, the percentage of organisms killed in all 3 set-ups was approximately the same For example, in the first set-up with the greatest number of bacteria, the control plate contained approximately 10,000 colonies, whereas in the plate poured at the end of two hours the number of colonies was reduced to 2500, or to 25 per cent of the control However, in the third

\*The progress of this investigation of immuno-transfusions is reported in the paper "Estimating the Increment in Bactericidal Power of Individuals' Blood Produced by Intravenous Injection of Typhoid Vaccine" by Charlotte Colwell and J L Yates

set up containing the smallest number of bacteria, there were 432 colonies in the control and 126 colonies in the two hour plate or a reduction to 29 per cent of the control. Therefore, in the first instance 7500 colonies disappeared, and although there were only 432 organisms in the second test, 29 per cent of these remained alive. These findings have been verified many times, and are entirely inexplicable at present. The experiments have been verified by using in different set ups with the same blood organisms which varied from about 100 in each 0.5 c.c. of blood to about 10,000, which is about the limit of the number that can be accurately counted on a plate. In each instance the percentage of organisms which disappeared, based on the number found in the control plate, was always approximately the same. The killing power of the blood in these in vitro tests does not seem to follow a quantitative law, but a law of percentages. It is interesting that Sir A. E. Wright with his slide cell method (of implanting and in culturing) found the same percentage relationships and absence of quantitative relationships. This will be discussed again later in this communication. We did not observe, however, the phenomenon which Wright called 'epiphyllaxis,' that is, a larger percentage of organisms being killed when a larger actual number are present in the blood bacterial mixtures. However his and our methods of testing this are not exactly comparable, since he used in culturing and we used explanting.

TABLE I

DIFFERENT NUMBERS OF BACTERIA IN SEVERAL TESTS WITH THE SAME AMOUNT OF THE SAME BLOOD. DEMONSTRATION THAT THE SAME PERCENTAGE OF ORGANISMS IS KILLED IN ALL OF THE TESTS, EVEN THOUGH FOR EXAMPLE MORE ORGANISMS WERE KILLED IN TEST 3 THAN WERE PRESENT ORIGINALLY IN TEST 5

DILUTION	CONTROL	2 HOURS INC	4 HOURS INC
1	Infinite number colonies		
2	Infinite number	Fewer	Even fewer
3	9760	2582 (26% of control)	208 (2.1% of control)
4	2276	66 (2.8% of control)	165 (7.4% of control)
5	432	126 (29% of control)	28 (6.4% of control)

We soon found that whereas we detected differences in the bactericidal power of different bloods duplicate set ups with the same blood sample and the same bacterial suspension gave results almost as different as obtained with different bloods. At this stage our results were sufficiently consistent to indicate that we were experimenting in the right direction but that our procedures needed refinement and standardization. We then investigated one possible variable after the other. We found that keeping the blood bacterial mixtures in a warm water bath increased the killing effect over that which occurred in the thermostat. We found that shaking the blood bacterial mixtures at frequent intervals increased the killing effect of the blood. This caused us to install a mechanical shaking device in the warm water bath. We found that there was less variation in duplicate samples when the blood was handled throughout in paraffin lined sterile containers. Replacing cotton stoppers by sterile rubber stoppers helped to give uniformity of results.

While we were in the midst of this standardization procedure we had the good fortune to show some of our results to Dr. Karl Kassowitz. We found

much to our pleasure, that he had been working on the problem of the bactericidal power of blood along similar lines, and was securing similar results. We had started our work independently, but he had been at work on the problem longer. He very kindly told us of an important difference between his test and ours. He was adding an appropriate number of bacteria to one sample of blood, and at hourly intervals was removing small samples for plating. He thereby obtained a sequence of results which could be charted as a curve. These curves of Dr. Kassowitz's showed that whereas some samples of blood might have the same quantitative killing effect on *Staphylococcus aureus* after a given interval such as two, three, or four hours, some bloods after that interval allowed the bacteria to increase in number, while others continued to kill the staphylococci for an additional period of time before the bacteria began to increase in number. It was evident that this method was an improvement on ours, and with Dr. Kassowitz's consent we adopted this modification. Dr. Kassowitz's extremely important results are reported in a paper by Drs. Gonce and Kassowitz,<sup>6</sup> which should be read by all interested in this field. Since the limited bibliography of this subject is discussed very completely in that paper, and since previously published methods for determining the bactericidal power of blood are criticized and reviewed, these will not be discussed here. It seems interesting that our results and our method, as finally evolved, should have paralleled so closely those of Gonce and Kassowitz. We do believe that we have paid more attention to refinements of the different procedures, and many times we have obtained duplicate results which check very accurately, almost as accurately as routine biochemical determinations. Two steps in our method seem to us to have essential and important differences from the test of Gonce and Kassowitz: one is that our blood-bacterial mixtures are shaken continuously in a warm water-bath, except for the extremely short time necessary to take samples; the other, which is possibly even more important, and which will be discussed later, is that we use heparinized blood, whereas they use defibrinated blood.

The following method is the one we now use and have used for the past year without change.

Dissolve 10 mg. of heparin in 1 cc. of physiologic saline, and sterilize in autoclave at 10 pounds pressure for ten minutes. One mg. of heparin will keep 5 cc. of human blood from clotting for twenty-four hours. Use approximately 0.1 cc. of solution for each 5 cc. of blood.

Withdraw the desired amount of blood aseptically from the patient's arm vein, and put it into a paraffin-lined glass container which holds the correct amount of heparin. Rotate gently to mix.

Centrifuge a twenty-hour broth culture of *Staphylococcus aureus* for three minutes at low speed to throw down large clumps of bacteria. Transfer with sterile pipette approximately 1 cc. of the top of the centrifuged culture to a clean sterile test tube. Shake thoroughly before using. Transfer 1 loopful of shaken culture to 50 cc. of sterile bouillon (loop should be between 3 and 4 mm. in diameter).

Melt tube of agar and cool to 50° C.

Transfer with 10 cc. pipette 4.5 cc. of heparinized blood into a small, paraffin-lined flask having a sterile rubber stopper (25 to 30 cc. flasks are most easily handled).

Transfer 0.5 cc. of the bacterial suspension to the 4.5 cc. of blood in the small flask. Tip flask and rotate to wash down from the sides any small drops of the bacterial dilution. Rotate gently to insure even distribution of bacteria.

Transfer immediately 0.5 cc of the blood bacterial mixture to the tube of melted cooled agar. Draw agar into the pipette and blow gently out again 3 times. Stir mixture with end of pipette. Blow out gently as much as possible of blood agar remaining in pipette. Pour plate, which serves as the control.

Place flask in shaking device in warm water bath (37° C). Remove 0.5 cc at two hour intervals for a period of eight hours and pour plates as for the control.

Incubate plates, and make accurate counts of colonies after twenty four hours.

**Results**—Only a few examples of results obtained in this investigation can be given here. Many checks and controls which were done cannot be mentioned. The data given are typical of tests repeated many times, with complete confirmation. Much of this work has been laborious and time consuming, but we have withheld its publication until we have convinced ourselves, after a year and a half of work, of the accuracy of the results.

Although the critical study of our own procedures has been a time consuming and complicated one, we can give assurance that the method, as finally evolved, is comparatively simple. It requires only a simple bacteriologic technique and the ordinary equipment of a bacteriologic laboratory, plus a mechanical shaker installed in a warm water bath. We have every reason to believe that with ordinary care our results can be verified.

TABLE II

BACTERICIDAL TESTS TO SHOW ACCURACY OF METHOD. BLOOD WAS TAKEN FROM A NORMAL INDIVIDUAL AT 3 DIFFERENT TIMES DURING THE DAY AND THE TESTS SET UP IN DUPLICATE. THE RESULTS CHECK WITHIN A FEW PER CENT.  
10 00 A M—W B C 5300

	CONTROL	2 HR		4 HR		6 HR	
A	3080	1344	- 43%	722	- 25 0%	Inf	number
B	3268	1224	- 37%	802	- 24 0%	Inf	number
Average	3174	1284	- 40%	802	- 24 5%		
1 30 P M							
A	2896	1178	- 40%	Lost		Inf	number
B	3160	1156	- 36%	796	- 20 0%	Inf	number
Average	3028	1167	- 38%			Inf	number
4 30 P M							
A	2912	1144	- 38%	696	- 23 0%	Inf	number
B	3184	1212	- 38%	848	- 26 0%	Inf	number
Average	3048	1178	- 38%	767	- 24 5%	Inf	number

Culture of *Staphylococcus aureus* eighteen hours old at 8 30. Centrifuged at low speed three minutes. 1 c.c. pipetted into clean sterile test tube shaken well and allowed to stand at room temperature. From this culture fresh suspensions were made every time the test was set up.

The accuracy of the method can be seen in the type experiment illustrated in Table II. Here blood was secured from the same individual at 10 A M, 1 30 P M, and 4 30 P M. Each time the test was set up in duplicate. The widest divergence in the measure of the bactericidal power at the end of two hours is 6 per cent, in the 10 A M test, i.e., the difference between 37 per cent and 43 per cent. The average of these figures, however, is 40 per cent which is a difference of only 2 per cent from the average of the results of the 1 30 P M and 4 30 P M tests. The results of the tests at the four hour period check even closer.

It seemed important to determine whether the bactericidal power of the blood resided mainly in the cellular elements or in the plasma. We found, as

illustrated in Table III, that heparinized plasma is either devoid, or practically devoid, of any bactericidal action on *Staphylococcus aureus*. The same is true of serum from defibrinated blood.

TABLE III

TEST DEMONSTRATING THAT HEPARINIZED PLASMA HAS NO BACTERICIDAL ACTION ON *STAPHYLOCOCCUS AUREUS*, WHEREAS THE WHOLE BLOOD FROM WHICH THE PLASMA IS OBTAINED HAS MARKED BACTERICIDAL ACTION

	CONTROL	2 HR	4 HR	6 HR
Whole blood - T	492	124 - 25%	96 - 19%	368 - 74%
Plasma - T	584	544	484	484
Whole blood - L	444	108 - 24%	60 - 13%	62 - 14%
Plasma - L	504	536	500	488

We next made many tests to determine, if possible, the manner in which the cellular elements of the blood removed staphylococci. This was done by removing small amounts of the blood-bacterial mixture immediately after it was made, then at frequent intervals, and later at longer intervals, making smears on glass slides and staining them with Wright's stain. With this stain both the blood cells and the bacteria stand out very clearly. Without such observations, the criticism might be made that the reduction in the number of colonies in the test is caused by the clumping of the bacteria, and not by their destruction. Table VII, we believe, demonstrates that the diminution in the number of colonies is because the cocci disappear by digestion and lysis in the leucocytes, and not because of clumping. It can be seen that in ten minutes phagocytosis has already begun, and in twenty minutes most cocci are within the polymorphonuclears. At the end of two hours practically no cocci are lying free. At four hours, when the maximum bactericidal action of the blood usually occurs in the test, there are fewer cocci present, and most of the polymorphonuclears contain only 2 or 3 cocci, instead of being loaded with them, as at the three-hour interval.

The tests of the kind just described had to be performed with the addition of a larger number of bacteria than was added when the ordinary test was performed. Ordinarily, the number of bacteria present was so small that it was difficult to find any in the small amount of blood on the smear. In some of these special tests, a comparatively large amount of a twenty-four-hour bouillon culture of *staphylococcus* had to be added to secure an adequate number of bacteria. In some of these special tests the leucocytes, after an hour or more, began to show degeneration and lysis, in all probability from lytic substances in the unusually large amount of added culture. Table VII represents one of the best of this group of experiments, and it was verified several times.

We next investigated whether the leucocytes decreased in number in the regular test where the usual small number of bacteria and small amount of lytic substances were added. We found, as illustrated in Table IV, by making leucocyte counts at two-hour intervals, that up to six hours there is practically no diminution in the number of leucocytes.

Because of suggestions in the article by Gonce and Kassowitz, and some of our own observations of marked bactericidal power of the blood of patients with a marked leucocytosis, we investigated the effect on the bactericidal

power of mechanically increasing the number of leucocytes in blood. This was done by securing an ample supply of heparinized blood and centrifuging most of it, removing the serum, and then sucking off with a pipette the layer of leucocytes on top of the layer of red cells. One sample of the original heparinized blood was tested and another sample was fortified with the leucocytes removed, and tested also. The result is shown in Table V. It will be seen that the leucocyte count on the original whole blood was 10,250 per c mm., and on the fortified blood 28,100 per c mm., and that the bactericidal power was markedly increased in the fortified blood.

TABLE IV

DEMONSTRATION THAT LEUCOCYTES DO NOT DIMINISH APPRECIABLY IN NUMBER DURING THE PROGRESS OF THE BACTERICIDAL TEST

	CONTROL	2 HR	4 HR	6 HR
White Blood Counts Whole Blood	9,530	11,750	9,200	8,000

LEUCOCYTE COUNTS WERE MADE IN THE BEGINNING AND AT TWO-HOUR INTERVALS, UP TO SIX HOURS WHEN THE PLATES WERE POURED FOR THE BACTERICIDAL TEST GIVEN BELOW

	CONTROL	2 HR	4 HR	6 HR
Whole Blood	100%	146 - 5%	410 - 15%	1760 - 63%

TABLE V

DEMONSTRATION THAT LEUCOCYTES ADDED TO WHOLE BLOOD INCREASE THE BACTERICIDAL POWER

	CONTROL	2 HR	4 HR	6 HR
Whole Blood Leuc. count - 10,250	2952	263 - 8%	382 - 12%	3200 - 100%
Whole Blood plus Leuc. Leuc count 28,100	2624	45 - 16%	21 - 0.8%	113 - 4.3%

Since our test was done with heparinized whole blood, and some of the methods reported, including that of Gonce and Kassowitz used defibrinated blood, it seemed important to compare the bactericidal action of blood prepared in these two ways. It will be noted also that the results of Gonce and Kassowitz show a continuation of the bactericidal action for a longer period of time than we found with our method. The results of one of these comparisons is found in Table VI. It will be seen that the bactericidal action of the

TABLE VI

DEMONSTRATION THAT DEFIBRINATED BLOOD HAS A GREATER BACTERICIDAL POWER THAN THE SAME BLOOD KEPT FLUID WITH HEPARIN

	CONTROL	2 HR	4 HR	6 HR
Hep <sub>1</sub>	2796	264 - 5.4%	221 - 7.9%	3680 - 131%
Hep <sub>2</sub>	2292	190 - 8.3%	160 - 6.9%	Inf number
Def	2290	23 - 1.0%	14 - 0.6%	7 - 0.3%

defibrinated blood is greater than that of the heparinized blood and continues for a longer time. The exact significance of this is not clear at present. We discovered by making blood counts that the process of defibrinating blood

caused marked reduction in the number of leucocytes In one instance this reduction was from about 7500 to 2500 per c mm

It seemed of interest to determine the effect on the bactericidal power of adding plasma from defibrinated blood to cells from heparinized blood, and plasma from heparinized blood to cells from defibrinated blood These results are shown in Table VIII It will be noticed that here also defibrinated whole blood had a stronger bactericidal action than heparinized whole blood, and that crossing the plasma with the cells in each direction gave mixtures whose

TABLE VII

STUDY OF DISAPPEARANCE OF COCCI FROM BLOOD BACTERIAL MIXTURE SMEARS MADE AT THE INTERVALS INDICATED WERE STAINED WITH WRIGHT'S STAIN AND EXAMINED MICROSCOPICALLY

TIME	MICROSCOPICAL EXAMINATION
Immediately	Many cocci, the majority occurring singly, few in pairs or in 3's or 4's, but never in large clumps
10 minutes	Cocci still very numerous Phagocytosis beginning Cocci grouped around and in polymorphonuclears for most part, although many cocci still occur singly
20 minutes	Few cocci lying free or singly Many are within polymorphonuclears
1 hour	Many cocci phagocytized
2 hours	Many cocci within the W B C Very few lying free
3 hours	Most polymorphonuclears loaded with cocci Occasional one seen in a lymphocyte
4 hours	Fewer cocci seen Most polymorphonuclears contain 2 or 3 diplococci, occasionally a polymorphonuclear is loaded with cocci
5 hours	Few cocci seen within the polymorphonuclears Occasionally a cluster lies free
6 hours	Polymorphonuclears seem to be disintegrating
7 hours	Many cocci lying free None of the few polymorphonuclears present are whole or healthy looking
8 hours	(Same picture as 7)
24 hours	Smear loaded with bacteria and debris which might be broken down W B C Only an occasional lymphocyte seen intact No polymorphonuclears

TABLE VIII

COMPARISON OF THE BACTERICIDAL ACTION OF 1 HEPARINIZED WHOLE BLOOD WITH DEFIBRINATED BLOOD, 2 HEPARINIZED PLASMA WITH DEFIBRINATED PLASMA, 3 HEPARINIZED PLASMA WITH CELLS FROM DEFIBRINATED BLOOD, AND 4 DEFIBRINATED PLASMA WITH CELLS FROM HEPARINIZED BLOOD

	CONTROL	2 HR	4 HR	6 HR
Hep Whole Blood	1314	162 - 12.3%	123 - 9.3%	5280 - 401%
Def Whole Blood	1492	50 - 3.3%	6 - 0.4%	440 - 29%
Hep Plasma	1860	1770	Inf number	Inf number
Def Plasma	1890	3120	Inf number	Inf number
Hep Plasma + cells from Def Blood	1468	49 - 3.3%	22 - 1.5%	2480 - 169%
Def Plasma + cells from Hep Blood	1392	56 - 4.1%	42 - 3%	920 - 66%

This test shows that

1 Defibrinated blood under the conditions of the test has a stronger bactericidal action against *Staphylococcus aureus* than heparinized blood

2 Plasma from defibrinated blood and plasma from heparinized blood show no bactericidal action

3 Cells from defibrinated blood to which heparinized plasma has been added show approximately the same bactericidal power as defibrinated whole blood

4 When plasma from defibrinated blood is added to cells from heparinized blood the bactericidal action is increased to about the same as defibrinated whole blood

If this test demonstrates anything it is that whereas plasma from defibrinated blood has no more bactericidal action than plasma from heparinized blood both cells and plasma from defibrinated blood when mixed with heparinized plasma and cells from heparinized blood respectively increase the bactericidal action of these mixtures

bactericidal power was approximately the same as that of defibrinated whole blood, and definitely stronger than that of heparinized whole blood. The cause of this action, which is different from what we expected, is entirely unexplained, and is well worth further study.

We have not investigated whether or not heparin has a deleterious effect on the phagocytic ability of the polymorphonuclear leucocytes. Whereas the amount of heparin used is very small, it might be that it diminishes the phagocytic ability of the leucocytes. This point, therefore, remains for further study. It is our impression, however, that the difference is not caused by the deleterious action of heparin, but by the liberation of some substance, in the process of defibrinating blood, which aids the bactericidal action. We confess that the results with defibrinated blood surprised us, as we expected that the bactericidal power would be diminished rather than increased. Defibrinated blood must injure some of the leucocytes, even though this injury is slight, and we expected this to have a deleterious effect. Whether defibrinated or heparinized blood gives more accurate results remains to be determined by further investigation. It seems to us that standardization of the technique can be more accurately accomplished by heparinizing the blood than by defibrinating it.

Practically all of our tests were done with *Staphylococcus aureus*. In a number of instances samples of the same blood were tested with *Streptococcus hemolyticus*. Not enough comparisons of this sort were made to be worth while reporting at this time. There are several results, nevertheless, of sufficient interest to mention. Although streptococci are somewhat more difficult to work with than staphylococci, the test can be made just as accurately. In the same blood the bactericidal action against these two organisms does not necessarily run parallel, and in fact is often widely divergent. Therefore the determination of the bactericidal power of a particular blood against one pyogenic organism does not indicate its bactericidal power against any other. In the hope that parallel results would obtain, and that results with pyogenic cocci could indicate the bactericidal power against tubercle bacilli, we tested out samples of blood from several patients with pulmonary tuberculosis (secured through the kindness of Dr. Kassowitz). We found that the bactericidal power of these samples against *Staphylococcus aureus* and against hemolytic streptococci was quite different, and no correlation whatsoever could be made between the clinical condition or the apparent resistance of the patients to the tuberculous infection and the results of our bactericidal tests.

In the presence of a streptococcus infection the blood of several patients showed a different degree of bactericidal action against streptococci and against staphylococci, usually more against streptococci than staphylococci. In the presence of *Staphylococcus aureus* infections, on several occasions we found that the patient's blood had a stronger bactericidal action against his own organism than against the stock organism. In this last instance two points are of interest: first, that the patient had a marked leucocytosis, and second, that the staphylococcus infection persisted for quite a while in spite of a marked bactericidal power of the patient's blood. One would have expected that, in the presence of such a strong bactericidal ability of the blood, the infection would clear up rapidly. The opposite conclusion, how



ever, seems the one indicated, that is, that even the strong bactericidal action which we found in vitro was not sufficient to eradicate rapidly the staphylococcus infection, and was probably only enough to prevent the patient from being overwhelmed by this infection

#### SUMMARY AND CONCLUSIONS

A method is described for determining the bactericidal power of blood against gram positive cocci, especially *Staphylococcus aureus*. This method is simple and gives results which check closely in duplicate tests. The method is therefore believed to be accurate. The results obtained with it should be of relative, if not of absolute, value.

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#### REFERENCES

- 1 Denison, Henry S. Amboceptors and Complement in Typhoid Sera, *Bull. Johns Hopkins Hosp.* 19: 263-268, 1908.
- 2 Heist, G. D., Solis Cohen, S., and Solis Cohen, Meyer. The Bactericidal Action of Whole Blood With a New Technique for Its Determination, *J. Immunol.* 3: 261-276, 1918.
- 3 Matsunami, T., and Kolmer, J. A. Relation of Meningococcal Activity of Blood to Resistance to Virulent Meningococci, *J. Immunol.* 3: 201-212, 1918.
- 4 Colebrook, L., and Storer, E. J. On the Reduction of the Bactericidal Power of Blood Which is Effected by Adding to It Citrate of Soda and Other Decalcifying Agents, and on the Question Whether Blood so Treated Should be Employed for Immuno Transfusion, *Brit. J. Exper. Path.* 5: 47-54, 1924.
- 5 Wright, Sir A. E., Colebrook, L., and Storer, E. J. New Principles in Therapeutic Inoculation, *Lancet* 104: 365-373, 417-420, 473-478, 1923.
- 6 Gonce, J. E., and Kassowitz, Karl. Action of Ultra Violet Irradiation on the Bactericidal Property of the Blood, *J. A. M. A.* 90: 280-284, 1928.

#### DISCUSSION

*Dr. Robert G. Maul*—It is very interesting to see the increasing phagocytes in the increase of leucocytes. I would like to ask if tests were carried out with the stock leucocytic extract.

*Dr. William Thalheimer* (closing)—That has not been carried out.

## THE INTERPRETATION OF THE WASSERMANN TEST\*

By B. MARKOWITZ, M.D., CHICAGO, ILL.

I SHOULD like to stress the importance of the proper interpretation which the clinician should make upon the complement fixation test, and as illustrations I will cite some of our personal cases and references found in medical literature. Much has been written on the Wassermann test; its technic is constantly improving, and we have today almost as many varieties in its performance as we have Wassermann workers. Its interpretation, however, has been slighted; the correlation of the reaction in the tube with the existing circumstances of the specimen and its host is not so frequent a topic of discussion.

The impression made by any statement depends upon the powers of perception or interpretation of the receiver. The impression made by the Wassermann test is dependent upon the interpretation placed upon its reaction by the serologist, and the interpretation placed upon its report by the physician. These interpretations are often the basis of considerable discussion, and are sometimes the cause of different impressions made by reports on the same specimen. This, together with the small percentage of error, which must be considered, does to some degree discredit the complement fixation test and may cause the clinician to give only half-hearted credence to the Wassermann report. For this reason I think that with a better understanding of the interpretation of the results found, and with a better understanding between serologist and clinician greater cooperation will be effected.

At this time, however, we will discuss only the clinician's interpretation of the serologist's report, and disregard the serologist's reading, excepting only that phase which influences the clinician's conception of the results of the Wassermann test. To do this we must first mention that even the Kolmer complement fixation modification considered by many the best of all Wassermann modifications is not technically specific since we cannot explain definitely why the complement becomes fixed in a positive serum. Weygandt, as far back as 1907, and Noguchi<sup>1</sup> since then, proved that positive results may be obtained in syphilitic sera by using antigens made of normal tissue. Kolmer<sup>2</sup> states that all we know of the syphilitic reaction is that a mixture of suitable extract plus a syphilitic serum is capable of fixing large amounts of complement. Davidsohn<sup>3</sup> states that the positive complement fixation test only indicates the presence of a syphilitic antibody in the blood of the patient at the time when the test was performed. Weil produced positive Wassermann reactions in the blood of rabbits which were injected with an alcoholic extract of rabbit organs, while Kolmer and Casselman<sup>4</sup> many years before showed

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that the serum of many normal rabbits reacted positively to the Wassermann test. As generally performed, therefore, the Wassermann test is not a true specific reaction, and if we want the clinician to properly interpret our reports, we must not only bear this nonspecificity in mind but impress him with its existence. We must educate him to the fact that this, in addition to further information of the Wassermann test, is necessary for the proper interpretation of its reaction.

Assuming though that our reaction is a true and specific one, we still find considerable controversy arising from the variations in laboratory reports on similar cases, and the clinician is often at a loss to know how to interpret them. The report he expects is probably one to be expected, but he fails to realize that not all cases respond serologically to the accepted rule. Seven exceptions are here discussed.

1 *Sudden Serologic Change in Treated Cases*—The sudden change in reaction of a treated case from negative to positive or vice versa is almost as well known as the chronicity of the disease itself. It frequently occurs that we report negative on a patient and two or three weeks later when the test is repeated, we find a two- or three-plus positive. To illustrate: Male, aged fifty-one, had been under treatment eighteen years ago. Three years ago he suddenly gave a four-plus reaction after constant negatives for a period of fifteen years. Since then he has been under constant treatment and observation. July, 1926, blood again became negative. Every three months a Wassermann was taken and always returned negative until September, 1927, when a two-plus Wassermann and two-plus Kahn were reported by two different laboratories. Just two weeks later, without any treatment, another Wassermann was done and this time reported completely negative by the same two laboratories. Why this sudden change occurs we do not know definitely, but as explained by Craig,<sup>5</sup> from these variations it is evident that the antibodies and lipo-trophic substances present in the patient's serum vary from day to day. Nichols<sup>6</sup> reported a case in which the patient, after two years of treatment, remained clinically and serologically negative for five years. After one injection of 0.6 gm. salvarsan, given as a provocative, his blood was negative daily for four successive days, but on the fifth day was reported four-plus positive.

2 *Serologic Variations in Untreated Cases*—Daily titration of the blood of untreated syphilitics shows that great variations may occur in the complement binding power of the serum of patients who receive no treatment. This variation may occur from day to day so that one or even several negative examinations in a suspected case should not always be considered sufficient evidence for excluding the disease. In a series of ten cases, all suffering from undoubted syphilis in various stages, Craig<sup>7</sup> has shown that some cases if tested on certain days gave a negative result, although serum from the same cases had previously been positive, and again became positive in a day or two. We have found one such variation in the case of a middle-aged woman who was an undoubtedluetie with a strongly positive Wassermann. She took only one injection of neosalvarsan and then disappeared for almost

a year Upon her return her serum was reported negative by the Wassermann test, and a repetition of the test three weeks later resulted in the same report Shortly after treatment was instituted, however, her serum again became positive We also found a similar variation in a case not presenting any signs, or giving any history of syphilis G M, female, widowed, aged forty two, one daughter aged fifteen She had a Wassermann taken only because her roommate was found to be luetic A history and physical examination were found negative for syphilis The Wassermann was reported four plus, the Kahn negative In view of the negative Kahn negative history and symptoms, the Wassermann was doubted and repeated one week later, at which time it was reported as a three plus Wassermann and again a negative Kahn Three days later the Wassermann was reported as a one plus and still negative on the Kahn Since then four Wassermann tests have been made on this patient and all found to be negative We must here call attention to the importance of these results in explaining why, in sending specimens from the same patient, but at different times to different laboratories, we find discrepancies between Wassermann reports We must therefore bear in mind and teach the clinician that when it is desired to obtain a report from more than one laboratory upon a suspected individual, the same specimen of blood should be used that is, obtain a sufficient quantity to be divided into two tubes and send one to each laboratory, instead of checking up at a later period on one test

3 *Negative Wassermann in Tertiary Syphilis*—Frequently we find a patient with a specific history of many years standing who presents definite symptoms of the disease and is pronounced clinically luetic A Wassermann test is done, and, much to the surprise of the clinician, the laboratory reports a negative reaction Two cases will illustrate L W, female aged forty nine gave a history of definite luetic infection at the age of twenty eight Was then given very vigorous treatment with mercury, and for a period of two or three years took a course of treatment each year and was pronounced cured She presented herself to her physician with a tumor mass about the size of an orange in the left groin He made a clinical diagnosis of sarcoma and removed a small piece which he sent to us for confirmation of his diagnosis Tissue examinations revealed central coagulation necrosis with a peripheral zone of granulation tissue rich in round cells, a Wassermann was suggested on the probability of this being a gumma Despite two negative Wassermann reports which we submitted, we suggested antiluetic treatment which we felt was justified in view of the positive history and the microscopic appearance of the tissue This course was followed and with the usual treatment of iodides salvarsan, and mercury, the mass was reduced to a small nodule about the size of a walnut After a three months rest following treatment the Wassermann test on this patient reacted four plus positive We, as serologists must in a case like this, be able to help the clinician give the patient some reasonable explanation why, despite definite symptoms the patient is serologically negative, and why, with treatment which reduced the symptoms the serum reacts positive after a rest period

The second case of this class, D M, aged fifty-four, druggist History of definiteluetie infection at the age of thirty with subsequent treatment over a period of three or four years Has since been free from any symptoms until several months prior to the time he presented himself for examination, when he noticed that his memory was failing His physician elicited definiteluetie symptoms and sent him in for a Wassermann test which was negative on three different occasions With a definite history and Argyll-Robertson pupils, we suggested a spinal fluid examination on which was returned a negative report on the Wassermann test, but a cell count of 34, globulin strongly positive, and a tabetic reaction in the Lange colloidal gold curve Despite the negative Wassermann a diagnosis of tabes was made, and the patient improved under antiluetic treatment Following a two months' course of treatment the patient was given a rest of six weeks, at which time his spinal fluid was four-plus positive, and the patient felt well

Solomon and Klauder<sup>s</sup> report similar findings of negative spinal fluid Wassermans in knownlueties, particularly in cases with vascular lesions Wynn<sup>o</sup> reports the disappearance of spinal fluid findings in cases of paresis, although symptoms continued

According to the statistics of Lundlan, only about 60 to 75 per cent of the cases of tertiary syphilis gave positive results In general paresis he reports the blood positive in 82 per cent of the cases, and the spinal fluid positive in practically 100 per cent Boas (quoted from<sup>1</sup>) reported that in early latent syphilis 40 per cent were found with positive reactions, in old latent cases he reported 22 per cent of properly treated cases and 74 per cent of insufficiently treated cases, as positive

As shown by these cases, the physician must not immediately convict the laboratory of poor work if the Wassermann report does not compare favorably with the clinical symptoms, but must bear in mind that tertiary syphilis will sometimes fail to give a positive Wassermann reaction

4 *Mild Positive Wassermans in Certain Nonluetie Individuals*—On the other hand the reverse condition is not infrequently found, a mild positive Wassermann in a nonluetie, as for instance in a case sent us B W, painter, middle-aged adult male with a score of chronic complaints, but no definite train of symptoms No specific history of any kind was given, and on examination there were no physical findings indicating a specific infection His mouth was in poor condition, teeth were carious, and there was a considerable degree of pyorrhea As a routine matter a Wassermann was done and returned from the laboratory as a mild positive The Wassermann was twice repeated and both times reported as a two-plus positive We suggested that the teeth and gums be treated Several abscessed teeth were extracted, and prophylactic treatment instituted Six months later the patient's physical condition was greatly improved and the Wassermann test returned negative on two different occasions This false reaction is not infrequently found in low grade systemic infections as illustrated The careful internist will not, in this case, make a diagnosis of syphilis on the strength of the Wassermann

alone, but will place the proper interpretation on the report submitted, and together with the history and physical findings, make a diagnosis other than syphilis.

The pathologic laboratories of the Mount Sinai Hospital at Philadelphia reports two cases in which weakly positive reactions were found in jaundiced patients. Corrigan<sup>10</sup> reported, on serum from 100 febrile patients, two strongly positive Wassermann reactions in cases of pneumonia with no syphilitic histories, and in one of these the Wassermann became negative after the crisis. One strongly positive reaction was found in a nonluetic case of endocarditis, and a weakly positive reaction was found in a case of epilepsy.

5 *Wassermann Fast Cases*—Another condition in which the Wassermann report does not compare favorably with the clinical course, is the so called Wassermann fast cases, in which the Wassermann reaction is persistently 100 per cent positive despite the repetition of vigorous courses of antiluetic treatment. I am mentioning the following case only because it differs slightly from the usual Wassermann fast case in that the serum of this patient became negative after the first course of treatment, and later again became and persistently remained strongly positive. Patient S. D. salesman male aged nineteen presented himself with definite secondaries and a four plus Wassermann in 1921. Following vigorous course of treatment, consisting of eighteen intravenous injections of neosalvarsan and forty intramuscular injections of mercury, one grain each, all given within a period of three months, his Wassermann came down to a one plus after two months rest. Instead of reporting for another course of treatment as he was instructed, this patient failed to return. A year later he returned and again his Wassermann was reported four plus. Since then he has been given many courses of treatment, including various preparations of salvarsan, mercury and bismuth, but his blood was persistently four plus positive. He has had to date over 100 intravenous injections of salvarsan, and about twice as many intramuscular injections of mercury and bismuth, with no serologic change.

Beinhauer and Jacob<sup>11</sup> think that in some cases the "Wassermann fast" syphilis is due to tissue saturation by the heavy metals used in the treatment. In this case however, we showed serologic progress after a very vigorous course of treatment, but after neglecting treatment for a year the serum became and remained strongly positive.

6 *Negative Wassermann in Primary Stage*—A negative Wassermann reaction, in the primary stage of syphilis is of no value whatever in eliminating the disease. Even if this negative reaction persists until the initial lesion disappears and during the stage preceding the onset of the secondary symptoms, it cannot be relied upon as evidence that syphilis does not exist, because the Wassermann test is frequently negative in the presence of an undoubted hard chancre. Bruck did report very early positive reactions in monkeys, even before the appearance of the lesion, but we are still to face the problem that some are negative long after the appearance of the lesion. Klauder and Kolmer<sup>12</sup> reported positive reactions in the secretion of the primary lesion, while the blood serum reacted negatively. According to statistics of Craig<sup>7</sup> reporting only strongly positive reactions complete in

hibition of hemolysis, in the first week after the appearance of the initial lesion, 14 per cent are positive, in the second week 22 per cent, in the third week 41 per cent and in the fourth week 53 per cent

Rosenau<sup>13</sup> states that the Wassermann reaction becomes positive by the tenth day in about 30 per cent of the cases, although it has occasionally been noted as early as three days after the initial lesion appeared. It is positive in 70 per cent by the end of the third week, and in 96 per cent of all cases by the fortieth day. That still leaves us a very large number of negatives in known positive cases. In our series of 42 cases, with no record of the age of the lesion, we found positive Wassermanns in only 24, or about 59 per cent.

*7 Wassermann Test Influenced by Ingestion of Alcohol*—That the ingestion of alcohol greatly influences the Wassermann reaction was definitely shown by Craig and Nichols,<sup>14</sup> who demonstrated by a series of nine cases that the positive reaction disappeared after its administration, and again reappeared after periods varying from several hours to several days. In these cases the blood was tested one hour before alcohol administration was begun, and one hour to several days after the last dose of alcoholic liquor had been administered.

In conclusion I would like to emphasize the following salient points:

1 The Wassermann test per se is not a truly specific test for syphilis, but rather an accidental finding, some, as yet unknown, biochemical change is present in the luetic individual, which causes the complement to become fixed.

2 Variations occur in the Wassermann reaction, depending upon the stage of the disease, early or late treatment, and individual influences produced by the host.

3 We must educate physicians to use the Wassermann test not as the main diagnostic factor in syphilis, but rather as one of the findings or symptoms of the case.

4 The proper interpretation of the report submitted by the serologist is the important factor in using the Wassermann test as an aid to diagnosis.

5 This interpretation, when properly understood, will effect greater cooperation between serologist and clinician.

#### REFERENCES

- 1 Noguchi, H. The Relation of Protein, Lipoids and Salts to the Wassermann Reaction, *J Exper Med* 11: 84-99, 1909.
- 2 Kolmer, J. A. A Practical Textbook of Infection, Immunity and Specific Therapy, Philadelphia and London, W. B. Saunders, pp. 899, 1915.
- 3 Davidsohn, I. The Laboratory Diagnosis of Syphilis, *Am J Syph* 12: 122-135, 1928.
- 4 Kolmer, J. A., and Casselman, A. J. Concerning the Wassermann Reaction With Normal Rabbit Serum, *J Med Res* 28: 369-375, 1913.
- 5 Craig, C. F. The Wassermann Test, St. Louis, C. V. Mosby Co., pp. 239, 1918.
- 6 Nichols, H. J. An Analysis of Anti-Typhoid Vaccines and a Consideration of Their Relative Practical Value at the Present Time, War Dept. (U. S.) S. G. O., Bull. No. 2, Wash., 23-36.
- 7 Craig, C. F. Variations in the Strength of the Wassermann Reaction in Untreated Syphilitic Infections, *J. A. M. A.* 62: 1232-1236, 1914.
- 8 Solomon, H. A., and Kluder, J. V. Neurosyphilis With Negative Spinal Fluid, *J. A. M. A.* 77: 1701-1706, 1921.

- 9 Wynn, J Paresis With Negative Spinal Fluid, *Am J Syph* 7 323 325 1923
- 10 Corrigan N Nonspecific Wassermann and Agglutinin Reactions With Serums From Patients With Febrile Diseases, *J Infect Dis* 41 457 460 1927
- 11 Beishauer, L G and Jacob F M Sodium Thiosulphate in Wassermann Fast Syphilis *Am J Syph* 12 61 66, 1928
- 12 Klauder, J V, and Kolmer, J A The Wassermann Test Performed With Chancre Fluid as an Aid to the Early Diagnosis of Syphilis, *Arch Dermat & Syph* 5 566 569, 1922
- 13 Rosenau M J Preventive Medicine and Hygiene, New York and London, D Appleton & Co ed 5, pp 1458, 1927
- 14 Craig C F, and Nichols H J The Effect of the Ingestion of Alcohol on the Result of the Complement Fixation Test in Syphilis a Preliminary Note *J A M A* 57 474 476, 1911

## PRESERVATION OF COMPLEMENT, A PRACTICAL STUDY\*

BY B W RHAMY, M D FORT WAYNE INDIANA

I<sup>1</sup> IN 1917 suggested the use of sodium acetate as a preservative of complement, setting forth that it would preserve complement a reasonable length of time, that it was not anticomplementary even in saturated solution, and that such preserved complement could be diluted with salt solution to any desired strength

In Kolmer's<sup>2</sup> classical study of the Wassermann test he compared several methods of preserving complement including sodium acetate. He concluded that 17 per cent sodium chloride yielded the best results. By this method 17 per cent sodium chloride was added to full strength guinea pig serum. For use 19 c c of distilled water were added to 1 c c of serum making a 120 dilution in isotonic salt solution. While I have adopted the Kolmer Wassermann technic with enthusiasm I was loath to discard the sodium acetate method of complement preservation, without first satisfying myself of the material superiority of salted complement. I therefore ran a parallel series, using both preparations of complements, over a period of three years on all Wassermanns coming into the laboratory. Pools were made from the total blood of four or five guinea pigs and the serum divided into two portions. To portion No 1 was added sodium chloride to make a 17 per cent mixture. To portion No 2 was added 6 parts of 12 per cent sodium acetate solution to each 4 parts of serum making a 40 per cent stock complement dilution containing 8 per cent of sodium acetate. This was placed in 5 c c vials and kept in the same compartment in the ice box with the salted complement.

These preserved complements Nos 1 and 2 were then used in the same dilution (120) in making the daily Wassermann tests but on alternate days, except that on days when unusual numbers of Wassermanns were run then the other complement was used on two successive days to keep the amounts equal, in order that one should not be exhausted before the other. Both complements were titrated the first day and after that on the days used. When either showed loss of fixability or tendency to false positives they were dis-

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carded Ox and human blood cells were used at different times, preserved with 0.125 per cent formalin. In this study, sharp changes in the unit strength of both complements will be noted, due to variations in the resistance (fragility) of different batches of red blood cells, as I had previously pointed out. The variations noted are mostly of lowered resistance of new batches of cells, since it was not practical, except in two instances, to distinguish whether an increase of amount of complement needed was due to loss of complement strength or to increased cell resistance. Considerable variation was noted in the unit strength from pigs obtained from different sources. One source of supply furnished pigs whose serum was weak in complement. Changing the source of supply resulted in getting a much better quality of complement.

In this study the main consideration was not so much how long complement was preserved, but rather the comparative behavior of the two forms of preserved complement made up from the same serum. For this reason the fact that the charts show samples of extremely poor complement, as well as good at all seasons of the year, makes the study all the more complete.

The number of days any one lot of preserved complement was in use depended on the following factors. First, the total quantity of any one lot of complement and its titer. Second, the volume of Wassermann tests to be done. Third, weather conditions, i.e.,

- a Season of the year (complement keeps best in cold weather)
- b Sudden sharp variations in barometric pressure sometimes caused sudden marked deterioration of preserved complement.

In charting this study I divided the series into the four seasons of the year, to show the effect of weather.

#### SERIES FOR THREE SPRINGS

The poorest lot of complement was good for seven days, the best for twenty-eight days, and the second best twenty-four days. Of 12 lots of complement used during the spring months, the average period of serviceability was sixteen days. In this group the units of both complements ran parallel with one exception. In the second lot the salted complement jumped from 0.35 c.c. to 0.45 c.c. on the eleventh day, then to 0.6 c.c. while the acetated complement did not go above 0.4 c.c. There were seven instances where new batches of cells had lower resistance than the previous batch, and therefore, required less complement. In this group the first drop in complement strength averaged at two and one-half days, the second drop at nine days.

#### SERIES FOR THREE SUMMERS

During the hot months both forms of complement deteriorated more rapidly and required for the three summers 19 lots of serum. Two lots were discarded after four days, while the best lot was in use nineteen days. The average period of use was ten days, the first drop in strength averaging at two days, the second at five days. In this group, the salted complement deteriorated more rapidly in one instance and the acetated complement in one instance, so in the entire group the honors were even. In six instances new

cells with lower resistance required less complement. In one instance, the third lot, new cells on the tenth day were of much greater resistance requiring twice as much complement.

#### SERIES FOR THREE FALLS

In this group showing nine lots of complement the poorest lot lasted seven days, the best twenty one days. The average period of use was fifteen days. The first drop averaged at three days, the second at eleven days. The salted complement deteriorated most rapidly in three instances, as against acetated complement once. Otherwise both complements ran parallel in quality. There were three instances where new cells of lower resistance required less complement.

#### SERIES FOR THREE WINTERS

In this group 11 lots of complement were used. Two lots lasted only seven days, while the best lot was used for thirty one days, beginning with a unit of 0.1 cc and finishing at 0.3 cc. The average period of use was nineteen days. There were nine instances where new cells of low resistance required less complement and one instance (the first lot of the group) where the opposite is very definitely shown. The unit on the first day was 0.75 cc but on the seventh day with new cells only 0.15 cc was required, finishing on the nineteenth day at 0.2 cc, proving it to be an excellent lot of complement. Both complements ran parallel in quality with two exceptions. In both instances it was the acetated complement that went bad. In this group there was no regularity of the first drop in complement strength. In seven lots it averaged on the third day while in four lots of exceptionally good complement it averaged at eleven days. The second drop averaged at eleven days. In the last lot there was no second drop of unit strength.

#### SUMMARY

A summary of these experiments indicates that during the spring months both complements were used from seven to twenty eight days, averaging fifteen days, during the summer months four to sixteen days averaging ten days, during the fall seven to twenty one days, averaging fifteen days, and during the winter seven to thirty one days averaging nineteen days.

There were 25 instances where the use of new batches of cells required a smaller complement unit, indicating that they had less resistance to hemolysis (greater fragility) than the previous batches. In two instances it was possible to note that new batches of cells showed definitely higher resistance to hemolysis. The first drop of complement strength ordinarily occurred on the second or third day, the second drop around the tenth day. A few lots of complement registered only the initial drop. Adult pigs furnished the best complement.

There were five instances in the whole series where the salted complement proved inferior and four instances where the acetated complement proved inferior. With this slight difference, neither form of complement proved materially superior to the other, unless the following propositions be considered exceptions.



- 1 Flexibility of dilution
- 2 Ease and simplicity of preparation
- 3 Retention of its preserving properties in any dilution, allowing closer titration

Having justified my faith in acetated complement, my next aim was to improve it. Sodium acetate has no bactericidal properties, 10 per cent solutions having no inhibitory action on the growth of streptococci. I have found that the temperature of the ice box depended on the uncertainties of the ice man. Sometimes it was full of ice but often nearly empty and occasionally entirely so, especially on a few very hot Sundays and holidays. At these times acetated complement was subject to deterioration from bacterial contamination. To prevent this I began adding three drops of toluol to each 5 c.c. of acetated complement. I have also increased the strength of the sodium acetate solution to 20 per cent and now make a 50 per cent stock complement dilution. Then about a year ago I got rid of the ice man problem by installing an ice machine. With this constant temperature plus the toluol and 20 per cent acetate solution the limits of usefulness of a lot of acetated complement is now apparently only limited by the total quantity at hand. As shown in Table I whatever amount was made up was used in its entirety, lasting on an average of about four weeks. The unit of complement at no time shows any wide variation from the first day's titration, averaging in the end about a half more. One lot of complement shown in Table II was made up Aug 4, 1927 and lasted until Sept 23 1927, a period of fifty two

TABLE II  
COMPLEMENT TITRATION BEGINNING AUGUST 4 1927

DAYS	AMOUNT IN MILS	DAYS	AMOUNT IN MILS
1	0.15	27†	0.1
2	0.15	28	0.1
3*	0.15	29	0.1
4	—	30	0.125
5	0.2	31	0.125
6*	0.2	32	—
7	0.2	33	0.15
8	0.2	34	0.15
9	0.2	35†	0.125
10	0.2	36	0.125
11	—	37	0.125
12	0.2	38	0.125
13*	0.2	39	—
14*	0.2	40	0.125
15	0.2	41	0.125
16	0.125	42	0.125
17	0.125	43	0.15
18	—	44	0.15
19	0.125	45	0.15
20	0.125	46	—
21	0.125	47	0.15
22	0.125	48	0.15
23	0.125	49	0.15
24	0.125	50	0.15
25	—	51	0.15
26	0.125	52	0.15†

Dilution 1:30 all other titrations done with 1:10 dilution

†New cells

‡All of this batch used up September 24th

days, giving sharp reactions to the end. The first dilution was 1-32, later 1-16. The unit on the first day was 0.15 c.c. (dilution 1-32), and on the fifty-second day it was just double or 0.15 c.c. of a 1-16 dilution.

#### REFERENCES

- 1 Rhamy, B. W. Preservation of Complement, *J. A. M. A.* 69: 973-974, 1917.
- 2 Kolmer, J. A., Matsunami, T., and Trist, Mary E. Studies in the Standardization of the Wassermann Reaction. V. The Preservation of Complement Serum, *Am. J. Syph.* 3: 513-540, 1919.

#### DISCUSSION OF PAPERS BY MARKOWITZ AND RHAMY

*Dr. H. C. Sweeney*—Regarding the complement fixation reaction in syphilis, we have made a few observations in our tuberculosis patients in Chicago, realizing that this reaction in certain cases is quite fleeting, so much so that before we permit the treatment of one of our tuberculosis patients we get two or three positive reactions on this patient before we send in the report. Now it seems to me that we must learn more of the physicochemical reaction taking place in the Wassermann before we should put too much stress on these borderline cases. It may be illustrated very well by what happens in tuberculosis. In this disease we have made an extensive study of the blood chemistry changes. We find that the albumin globulin ratio varies many times quite abruptly in a very short period of time, so that certain physicochemical reactions taken from week to week will vary a good deal. It seems quite reasonable therefore, that the same thing may happen in syphilis except there is a definite relation there of the proteins to the disease. Until we know more of the chemistry of this reaction we must be careful in our evaluation of this test. Clinicians must also be taught to interpret the laboratory man's report.

*Dr. J. J. Moore*—Dr. Markowitz's paper is very important. It is very disconcerting for a clinician to send separate samples of the same blood to three laboratories approved by the American Medical Association, directed by members of this Society and get three different reports. It seems to me that we have not gone far enough in our cooperation with each other in seeing that our reports agree. In the last year in Chicago our laboratory and another approved laboratory have run a number of Wassermanns upon the same individuals with no agreement. Fortunately for us since our reactions were positive, all these cases were old treated luetics. Another approved laboratory has agreed consistently with us for the last three years. We have now suggested that those directors of approved laboratories in the same localities get together and find out what is wrong with their Wassermanns. Many clinicians at the present time get up and say the Wassermann is of no value whatever. I would not take any four plus Wassermann as a diagnosis of syphilis unless it was backed up by other reports or clinical symptoms. We can get together and get our antigens so fixed up that the laboratories in the same community can agree more closely on bloods taken on the same patient and sent to different laboratories.

*Dr. Wm. G. Exton*—Some of you will remember that in the symposium we had at San Francisco this same topic was thoroughly discussed, and that I brought to your attention the fact that insurance offices are constantly puzzled by the discrepancies reported from the laboratories in the different parts of the country which do Wassermann tests. I think that the crux of the situation to some extent at least, lies in the fact that not only are different methods used but that when they do the same test, such as Kolmer's technic, each has some different little quirk that may bring its work out of line with the others. Personally, I regard the whole matter as a positive scandal which is going to hinder clinical pathology if some way is not found to get uniform results from different laboratories.

*Dr. Frederic E. Sondern*—Dr. Moore's and Dr. Exton's statements are well founded. They are brought about by various conditions as the reader of the first paper indicated in closing. Communications of this kind are of value chiefly to the clinician. We are apt to talk about these things among ourselves but often fail to go before the clinician's societies and talk in the same way, which is our duty as the clinician is often not sufficiently informed on the very things that we are now considering. The one great difficulty is the variation

in reaction of people that have been treated for syphilis Dr Markowitz spoke of several conditions in which a patient was negative one day and positive later on and negative again. It brought to my mind one instance that I experienced. In this case my reaction was completely negative and that of another laboratory was completely positive. The blood for these tests was not taken at the same time but on the same day. They were repeated one week later and this time my test was completely positive while the other was completely negative. Subsequent investigation proved that the two negatives were taken in the afternoon and the two positives in the morning, and we also found that it was the patient's habit to drink three high balls at noon. If Dr Markowitz's paper were read to a society of clinicians I think it would do a great deal of good.

The question of nonspecific positives is also important. The seriousness of a false positive reaction affects the private laboratory worker to a greater extent than the serologist of a large institution. In the latter the tests are more frequently repeated and the error is generally discovered more quickly. If the result of the Wassermann test is used conjointly with clinical manifestations in diagnosis and prognosis harmony generally results, but if the result of the Wassermann test is the only factor used for this purpose and particularly if the patient is taught to follow his own case on the basis of Wassermann reports, only then it is not unusual to have a confused clinician and a doubting patient.

*Dr A H Schade*—Dr Sondern has hit the nail on the head. In Toledo the pathologists attempt to get a paper before the academy of medicine at least once a year on the interpretation of the Wassermann test, stressing that the Wassermann test is only one of the symptoms of syphilis.

*Dr B Markowitz*—I have not much to add except that as Dr Sondern has pointed out it is very important to educate the physician on this point of Wassermann reaction. I think we should take it upon ourselves as Dr Moore says, that every one of us should go back to his locality and get together with other pathologists in the community and decide upon a technique that would come at least close to being the same. We should attempt in that particular community before the different medical societies, to point out that idea of false positives in syphilis.

*Dr A H Sanford*—It seems to me that Dr Rhamy's paper should have some discussion. Just one question he talked about using new cells. I am not quite clear as to what he uses. Do you use pooled cells from the slaughterhouse? Do you take one sheep and bleed him or do you use a number of sheep?

*Dr B W Rhamy*—I used both ox and human cells preserved in 0.125 per cent formalin. When human cells were used a pool was made from whatever fresh cells were available in the samples at hand for Wassermann tests. These suspensions were used for about three days then a new supply made up.

*Dr A H Sanford*—The reason I asked was that we have had the experience that when we used one sheep right along, the cells will finally become resistant from that sheep so that we have to get a new one. We find that there is quite a little variation in sheep cells when we start in using new sheep.

*Dr B W Rhamy*—I had one batch of complement, whose titer on the first day it was titered, was 0.75 c.c. of a 1:16 dilution. Four days later with a new batch of cells it was 0.15 c.c. The cells that were used the first day were evidently of extremely high resistance. They were ox cells and were hard to separate from the serum.

## THE SEDIMENTATION TIME OF BLOOD IN JAUNDICE\*

BY N ROSENTHAL, M D , AND M I BLOWSTEIN, M D , NEW YORK

**J**AUNDICE is a symptom which may result from numerous factors, these are sometimes obvious, although often obscure. The diagnosis of certain forms of hemolytic jaundice, such as pernicious anemia and congenital, splenomegalic, acholuric jaundice, can readily be made from the blood picture and the fragility of red blood cells. In cases of cholelithiasis, new-growths, catarrhal jaundice and cirrhosis of the liver accompanied by jaundice, however, the problem is more difficult, no definite laboratory finding being considered diagnostic in these conditions. Nevertheless, the intensity of the jaundice may be determined by the icterus index, the possible extent of liver damage may be ascertained in cases of nonobstructive jaundice by the bromsulphthalein test (S Rosenthal<sup>1</sup>), and the degree of obstruction in bile ducts or liver cells may be estimated from examinations of the urine and stools for urobilin, bile and color. Complete obstruction of the bile ducts or in the liver cells is shown by the absence of urobilin in the stools and urine, and by the passage of clay-colored stools. Again, the presence of leucin and tyrosin in the urine are to be regarded as important findings, being indicative of liver degeneration.

Recently the sedimentation reaction of red blood cells was proposed as an additional aid in the differential diagnosis of certain cases of jaundice. As the conclusions reported by Katz and Leffkowitz<sup>2</sup> concerning the sedimentation rate in jaundice were based on comparatively few cases, it was believed that a more extensive and systematic study of this reaction might more fully determine its extent or limitation. With this purpose in mind, observations were made by us in a series of 69 cases, in which a definite diagnosis was made by operation, x-ray, autopsy, or clinical observation, especially in cases of catarrhal jaundice and cirrhosis of the liver.

### METHODS EMPLOYED

The Linzenmeier<sup>3</sup> method for the sedimentation rate was employed in the present series. For this purpose citrated blood was used in a proportion of one part of 5 per cent sodium citrate to four parts of blood. The sedimentation time in the usual Linzenmeier tubes was noted when the red blood cells reach the 18 mm mark. The normal time with this method was 180 minutes or longer. For the determination of the icterus index, we used the Brown<sup>4</sup> modification of the Meulengracht<sup>5</sup> reaction. Our experiences were similar to those of Shattuck, Killian, and Preston,<sup>6</sup> regarding the dependability of the icterus index. The direct reading of the color of the serum or plasma was more sensitive than the Van den Bergh reaction in milder grades of jaundice.

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<sup>1</sup>Read before the Seventh Annual Convention of the American Society of Clinical Pathologists Minneapolis Minn June 8 9 and 11 1928

## EFFECT OF JAUNDICE ON THE SEDIMENTATION RATE (CHAPT 1)

About 80 per cent of our cases accompanied by jaundice showed a sedimentation time which was less than normal. Most of these cases were rapid, between eleven and eighty minutes. The sedimentation rate bears no relation to the degree or duration of jaundice or of bile obstruction. These results seem to be rather paradoxical. According to the experiments *in vitro* by Johannes and Joseph Vorschütz,<sup>7</sup> and by Katz and Radt,<sup>8</sup> bilirubin itself does not influence the sedimentation rate but bile salts have a marked inhibitory effect. Cholesterol according to Kurten<sup>9</sup> hastens, and lecithin inhibits the sedimentation rate of the red cells. The fibrinogen and globulin of the blood, in jaundice, as reported by Starlinger,<sup>10</sup> seem to be increased except in grave icterus where the fibrinogen is greatly diminished, as pre-

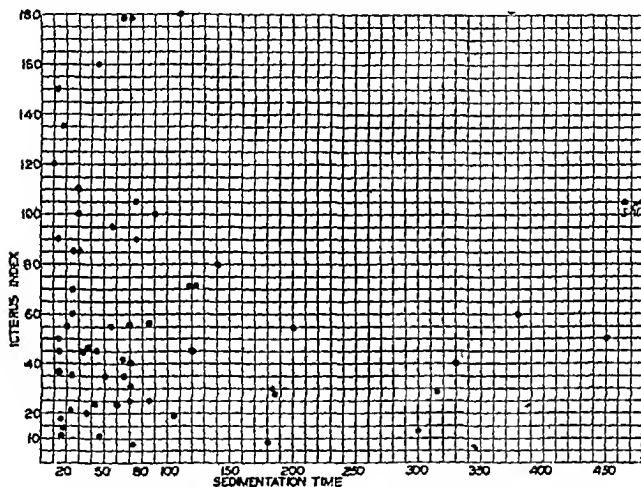


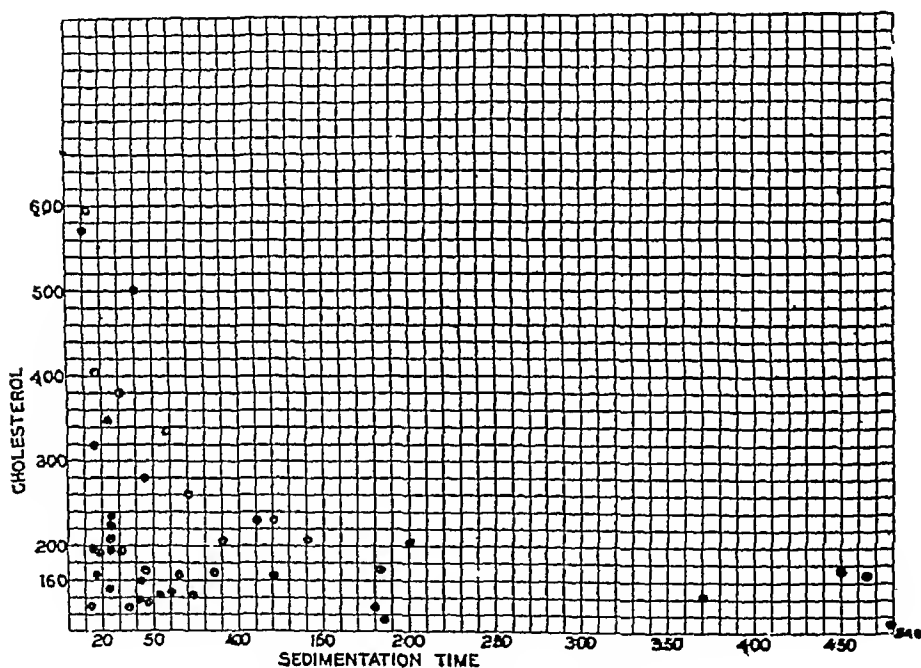
Chart 1.—Corresponding values of icterus index and sedimentation time.

viously shown by Whipple,<sup>11</sup> Gram,<sup>1</sup> and others. Synergistic and antagonistic elements which affect the sedimentation rate are therefore present in icteric plasma. The elements which inhibit the sedimentation of the red cells as the bile salts, are increased in practically all cases of jaundice, according to the observations of F. Rosenthal and Wislicki,<sup>12</sup> and Shattuck, Katayama, and Killian<sup>14</sup>, and the lipin phosphorus (lecithin) is increased in obstructive jaundice (Byrom and Kay<sup>15</sup>). In spite of the presence of these inhibitory factors in icteric plasma the sedimentation rate in most cases of jaundice shows a marked tendency to increase. Fibrinogen and globulin may influence but do not show any constant correspondence to the sedimentation rate, as found by Fahraeus<sup>16</sup> and later by Pinner.<sup>17</sup> Conclusions however which do not agree with those reached by Gram,<sup>18</sup> Frisch and Starlinger<sup>19</sup> on the relation of



fibrinogen to the sedimentation rate. The effect of jaundice on the dispersion state of globulin which is believed to be the main factor in the sedimentation of the red blood cells, is problematical.

Johannes and Joseph Vorschütz<sup>7</sup> state that jaundice of short duration does not affect the sedimentation rate, but that jaundice of long standing slows the rate. They attribute this action to the increase of bile salts and lecithin. Katz and Radt<sup>8</sup> confirm the observation of the effect of bile salts in vitro and believe that the retention of bile salts affects the sedimentation rate in cases of jaundice. On the other hand, Lohr<sup>20</sup> found a rapid sedimentation time in cholelithiasis with or without jaundice, but in such cases Shattuck, Katayama, and Kilhan<sup>14</sup> have shown a marked retention of bile salts in the blood.



found to be rapid. It would thus appear that a greatly increased cholesterol of the blood is an important factor in the sedimentation of red cells in cases of jaundice.

#### SEDIMENTATION RATE IN VARIOUS DISEASES ASSOCIATED WITH JAUNDICE

1 *Carcinoma* (Table I) —In a series of 18 cases of newgrowth accompanied by complete or nonobstructive jaundice, proved by autopsy, x ray or operation, the sedimentation rate was rapid in all except two cases. In one case, the sedimentation time was normal (185 minutes and, later, 315 minutes). This patient presented a moderate degree of jaundice (icterus index 28) and a definite polycythemia which was responsible for the slow sedimentation rate. The

TABLE I  
CARCINOMA

CASE	S.R.	L.F.T. %	II	CHOL	COMMON DUCT OBSTRUCTION	REMARKS
1 K.H.	11	100	150		Complete	
2 S.B.	18	25	12	182 mg	None	
3 H.H.	25	70	70	224 mg	Complete	Ca Pancreas
4 S.B.	25	60	60	248 mg	Partial	Anemia
5 I.G.	25	35	90		Complete	Metastatic Ca
6 F.S.	30	60	85	380 mg	Complete	Sarcoma of Intestines
7 S.B.	30	100	110	230 mg	Complete	Ca of Common Duct
8 M.P.	30	70	100	182 mg	Complete	Ca Papilla
9 A.S.	33	40	45	216 mg	Complete	Ca Stomach
10 E.T.	35	25	30		None	Metastatic Ca
	25	35	18		None	
11 H.M.	38	40	46	130 mg	None	Metastatic Ca of Liver
12 B.G.	58	40	95	340 mg	Complete	Ca Pancreas
13 D.G.	70	70	45		Complete	Ca Pancreas, Anemia
14 I.G.	85	85	56	176 mg	Complete	Ca Common Duct
15 M.S.	110		180	230 mg	Complete	Ca Pancreas
16 S.A.	120	70	45	164 mg	None	Metastatic Ca of Liver
17 J.S.	185	50	28	124 mg	None	Hypernephroma
	315	35	28			Thrombosis of Hepatic veins Polycythemia
18 F.A.	360		15	160 mg	None	Metastatic Ca

S.R.=Sedimentation rate minutes

L.F.T.=Liver function test.

II=Icteric Index.

Chol.=Cholesterol

postmortem findings showed a hypernephroma of the kidney, with involvement of the vena cava and thrombosis of the hepatic veins. No metastases were present in the liver. Blood concentration in the other case of carcinoma and jaundice probably influenced the sedimentation rate. All patients were afebrile, with the exception of Case 2. The cholesterol findings were normal except in Case 9, this patient also having diabetes.

These results agree with the findings of Fahraeus,<sup>10</sup> Kovacs,<sup>21</sup> and Rubin, in cases of carcinoma with or without jaundice.

2 *Catarrhal Jaundice* (Table II) —Catarrhal jaundice is not a disease entity. It consists, possibly, of two groups, one of which has a predominating infectious element (cholangitis), which may be designated as infectious hepatitis. The sedimentation rate is rapid in such cases. The other group begins insidiously without any infectious element. The term hepatosis, sug-

gested by Geronne,<sup>23</sup> is more appropriate for this particular condition, indicating a parenchymatous change of the liver cells, which was found by Eppinger,<sup>24</sup> Klemperei,<sup>25</sup> and others. In such cases leucin and tyrosin are occasionally present in the urine. Hepatosis not only occurs in young adults, but may also appear in later life, at which time it is difficult to differentiate from carcinoma with involvement of the liver bile ducts. The disease is not usually fatal, in a few cases, however, the lesions are so extensive that acute or subacute yellow atrophy of the liver may supervene. The etiology of the condition is still obscure. Sixteen cases of our series showed marked variations in the sedimentation rate. The sedimentation time was rapid in seven cases and normal or somewhat delayed in nine others. Apparently the presence or absence of bile in the stools does not affect the sedimentation rate. The cholesterol content of the blood was normal except in Case 1. This particular patient showed a hypercholesterolemia, secondary anemia, and diabetes mellitus. The jaundice subsided later.

TABLE II  
HEPATOSIS (CATARRHAL JAUNDICE)

CASE	SR	LFT %	II	CHOL	COMMON DUCT OBSTRUCTION	REMARKS
1 M A	18	100	135	412 mg	Complete	Secondary anemia, amyloidosis
	10	100	120	570 mg	Complete	Two weeks later
	85	15	25		None	Two months later
2 M H	30	10	45		None	
3 E M	43	45	45	140 mg	Complete	
4 P T	45	90	160	170 mg	Complete	
	70		32		None	Three weeks later
5 A S	60	15	24	148 mg	None	
6 B H	65	45	35	164 mg	None	
7 F S	90	80	100	208 mg	Complete	
8 A S	110	35	30	230 mg	Complete	
9 N R	120	70	72		Complete	
10 M C	200	50	55	208 mg	Complete	
11 M C	210	100	60	154 mg	Complete	
12 A W	300	100	122		None	
	215	40	80	260 mg	None	Nine days later
13 L S	330	60	40		Complete	
14 C S	370	70	60	142 mg	Complete	Pregnancy
15 W C	450	70	50	176 mg	None	
16 A A	465	45	110	181 mg	None	

Gram<sup>12</sup> reported a normal sedimentation time and normal blood fibrin in a case of catarrhal jaundice. Vorschütz<sup>26</sup> found an increased sedimentation rate in three cases of catarrhal jaundice (one to two hours). Kovacs<sup>21</sup> noticed a normal or slow sedimentation rate in four cases of catarrhal jaundice. He suggested the sedimentation time as a valuable diagnostic aid, as catarrhal jaundice shows a normal sedimentation rate and carcinoma with obstruction of the common duct shows a rapid rate. According to our results we cannot consider a rapid sedimentation rate as sufficient to exclude catarrhal jaundice but believe that a normal or slow sedimentation rate is suggestive of this condition.

3 *Cirrhosis of the Liver* (Table III).—In cirrhosis of the liver the sedimentation rate shows marked variations. In nine of our cases there was in-

creased sedimentation of the red cells and in three the rate was normal. The importance of the clinical course and other blood findings in these cases must not be disregarded, nor should too great reliance be placed on the sedimentation rate which may be modified by the possible presence of anemia. The bromsulphthalein liver function test offers considerable information concerning the condition of the liver, especially with a low icterus index. The high cholesterol content of the blood, in Case 1, was associated with amyloidosis and tuberculosis of the lymph nodes. An exploratory operation on this patient showed a moderate cirrhosis of the liver. The cases of splenomegalic cirrhosis of the liver and Banti's disease had the characteristic blood picture of anemia, leucopenia and thrombocytopenia. The sedimentation time was more rapid in cases with a severe anemia. Other cases of cirrhosis of the liver without jaundice were not included in this group, but their sedimentation time was observed to be normal.

TABLE III  
CIRRHOSIS OF LIVER

CASE	S.R.	L.F.T %	LI	CHOL	COMMON DUCT OBSTRUCTION	REMARKS
1 M.S.	11	100	50	568 mg	None	Tuberculosis of lymph nodes
2 T.M.	11	30	27	169 mg	None	Splenomegalic cirrhosis
3 H.S.	15	65	75	356 mg	None	Severe anemia Banti's Disease
4 R.C.	17	8	14	164 mg	None	Splenomegalic cirrhosis
5 A.S.	24	30	35	356 mg	None	Atrophic cirrhosis anemia
6 M.K.	48	10	12	136 mg	None	Atrophic cirrhosis ascites
7 T.B.	62	90	77	240 mg	None	Atrophic cirrhosis of the liver
8 F.W.	70	100	180	260 mg	None	Atrophic cirrhosis
9 A.P.	72	20	7	142 mg	None	Ascites severe anemia
10 S.P.	180	20	9	136 mg	None	Atrophic cirrhosis ascites
11 R.M.	182	60	30	176 mg	None	Atrophic cirrhosis ascites
12 A.B.	300	35	13		None	Banti's Disease

4 *Lues* (Table IV) —Determinations of the sedimentation rate in cases of luetic hepatitis in the secondary stage were not made in our series, but, according to the observations of Klopstock<sup>27</sup> these cases show a rapid sedimentation time. The cases of lues under our observation gave a definite history of long continued salvarsan treatment prior to the onset of the jaundice. We found the sedimentation time to be rapid in four out of six subjects. In one the sedimentation time was markedly increased on account of an unusual com-

TABLE IV  
LUES

CASE	S.R.	L.F.T %	LI	CHOL	COMMON DUCT OBSTRUCTION	REMARKS
1 D.A.	13	65	90		None	Schistosomiasis of liver Post salvarsan
2 A.K.	54	70	55	142 mg	Complete	Post salvarsan
3 N.H.	75	70	105		Complete	Post salvarsan
	55	90	182		Complete	One month later
	39	5	20		None	Six weeks later
4 S.F.	70	50	40	240 mg	None	Post salvarsan
5 H.S.	140	90	80	208 mg	None	Post salvarsan
6 A.M.	540	90	105	126 mg	Complete	Post salvarsan

phleation of schistosomiasis of the liver Klopstock reported a normal sedimentation rate in two cases of hepatitis following salvarsan administration

5 *Miscellaneous Cases* (Table V) —The sedimentation rate was found to be increased in all cases complicated by infection or anemia In Case 12 a case of polycythemia vera complicated by jaundice and anemia as a result of the use of phenylhydrazine, the sedimentation rate increased Previously, the sedimentation rate in this patient was extremely slow, the red blood cells having sedimented about 5 mm after two days Case 16, a man of sixty-two, showed marked jaundice which at first appeared to be due to carcinoma Leucin and tyrosin were found in the urine, and it was suspected that marked

TABLE V  
MISCELLANEOUS CASES

CASE	SR	LFT %	II	CHOL	COMMON DUCT OBSTRUCTION	DIAGNOSIS
1 EH	11	20	36	130 mg	None	Pregnancy, toxic hepatitis Chills and fever
2 NK	14	35	45	320 mg	Partial	Stricture of common duct
3 ML	15	0	18	190 mg	None	Anemia, thrombosis of portal vein
4 IS	16	15	12	320 mg	None	Cholecystitis
5 HM	20	50	55		None	Lymphatic leukemia
6 DS	23	10	21	156 mg	None	Cholangitis Chills and fever
7 AA	25	65	85	190 mg	Incomplete	Anemia
8 BP	28	85	45	260 mg	Complete	Cholangitis
9 AL	42	0	24	160 mg	None	Common duct stone
10 SS	45	15	35	280 mg	None	Acute rheumatic fever
11 CS	65	45	52	80 mg	None	Cholelithiasis
12 BR	70	5	25		None	Cardiac decompensation
13 HT	75	85	90		Complete	Polycythemia, jaundice following phenylhydrazine
14 HL	95	40	20	260 mg	None	Common duct stone
15 CO	105	15	18		None	Leukemia
16 JW	110	70	100	160 mg	None	Cardiac decompensation
17 ED	120	70	72	230 mg	Partial	Subacute yellow atrophy 2 weeks later
						Stricture of common duct

liver degeneration was present Although his general condition became worse, the icterus index and liver function test showed some improvement This indicated that we were not dealing with a carcinoma The sedimentation rate also became more rapid (110 minutes to 38 minutes) The patient died, and at autopsy a subacute yellow atrophy of the liver was found In two cases of common duct obstruction due to stone the sedimentation rate was found to be rapid

#### SUMMARY

As shown by the following résumé of our cases, there is but slight variation in the sedimentation time of the various groups in which jaundice was present

1 Catarrhal jaundice (16 cases) Sedimentation time 18 minutes to 465 minutes

2 Carcinoma and jaundice (18 cases) Sedimentation time 11 minutes to 360 minutes

3 Cirrhosis of the liver (12 cases) Sedimentation time 11 minutes to 300 minutes

4 Lues and jaundice (6 cases) Sedimentation time varies from 13 minutes to 540 minutes

5 Miscellaneous (17 cases) Sedimentation time 11 minutes to 120 minutes

It therefore seems evident that the sedimentation reaction is only of value in certain cases of jaundice provided other data are available, such as the degree of liver impairment as determined by the bromsulphthalein test, intensity of the jaundice, and the presence of common duct obstruction or bile retention (as in hepatosis). The presence of bile in the urine and observation of the color of the stools are important factors in determining the existence of complete or partial obstruction of the bile ducts. The symptoms of the patient and the clinical course are considerations which must greatly influence the diagnosis.

The general variations of the sedimentation time are about the same in all varieties of jaundice. Comparable variations of the icterus index, cholesterol, and the results of liver function tests with bromsulphthalein can be noted in the various groups (see tables). The sedimentation time in catarrhal jaundice tends to be slow and in carcinoma with jaundice, it tends to be rapid in the majority of cases. This is an important differential diagnostic point in cases of afebrile jaundice when either condition is suspected.

The underlying factors which influence the variations in the sedimentation time have not been determined. The experiments *in vitro* with bile salts do not correspond with the sedimentation rate of red cells in icteric plasma. Even the relation of fibrinogen and other proteins to the sedimentation reaction of icteric blood is not definite, as certain cases of acute and subacute yellow atrophy of the liver which is accompanied by a marked diminution of the fibrinogen, have shown a rapid sedimentation time of the red cells. The factors which modify the sedimentation rate in icteric plasma require further study.

#### CONCLUSIONS

1 The sedimentation time of the blood is not a specific diagnostic or prognostic procedure in diseases accompanied by jaundice.

2 The majority of cases with jaundice show an increased sedimentation rate of the red blood cells.

3 Subnormal or normal variations of the blood cholesterol apparently have no effect on the sedimentation rate, but hypercholesterinemia (over 300 mg) is accompanied by an increased sedimentation rate.

4 The sedimentation time in most of the cases of carcinoma and cholelithiasis with or without obstruction of the common duct is rapid. A normal sedimentation time in a jaundiced patient (without polycythemia) is suggestive of hepatosis (catarrhal jaundice). In other conditions of the blood and liver accompanied by jaundice, the sedimentation time shows marked variations. These depend upon infection, anemia and other unknown factors.

## REFERENCES

- 1 Rosenthal, S M, and White, E C Clinical Application of the Bromsulphthalein Test for Hepatic Function, *J A M A* 84 1112 1114, 1925
- 2 Katz, G, and Leffkowitz, M Die Blutkörperchensenkung, *Ergebn d inn Med u Kinderh* 33 266 392, 1928
- 3 Lunzenmeier, G Kritisches Sammelreferat über die Blutkörperchensenkungsgeschwindigkeit, *Deutsche med Wchnschr* 48 1023 1024, 1922
- 4 Brown, A L Rapid Clinical Method for the Determination of the Icterus Index, *Arch Path* 3 409 410, 1927
- 5 Meulengracht, E The Clinical Importance of the Search for Bile Pigment in the Serum, *Ugesk f Læger* 81 1785 1799, 1919
- 6 Shattuck, H F, Kilham, J A, and Preston, M A Comparison of Quantitative Methods for the Bilirubin of the Blood, *J LAB & CLIN MED* 12 802 810, 1927
- 7 Vorschütz, Johannes, and Joseph Die Bedeutung der Hamagglutination und Bakterienagglutination als Diagnostikum und ihre Erklärung, *Mitt a d Grenzgeb d Med u Chir* 34 662 677, 1922
- 8 Katz, G, and Radt, P Die Blutkörperchensenkung beim Ikterus, *Med Klin* 23 760 761, 1927
- 9 Kurten, H Die Senkungsgeschwindigkeit der roten Blutkörperchen in ihrer Beziehung zu Cholesterin und Lecithin, *Pflug Arch f d ges Physiol* 185 248 261, 1920
- 10 Starlinger, W Ueber das Verhalten der zirkulierenden Eiweisskörper des menschlichen Plasmas unter normalen und krankhaften Bedingungen (Untersuchungen über Verteilungsverhältnis und physikochemische Konstanten) *Deutsche med Wchnschr* 54 731 733, 1928
- 11 Whipple, G H Fibrinogen I An Investigation Concerning Its Origin and Destruction, *Am J Physiol* 33 50 69, 1914
- 12 Gram, H C The Results of a New Method for Determining the Fibrin Percentage in Blood and Plasma, *Acta Med Scandinav* 56 107 161, 1922
- 13 Rosenthal, F, and Wislitzki, L Gallensauren Studien am ikterischen Menschen, *Klin Wchnschr* 6 781 784, 1927
- 14 Shattuck, H F, Katraman, I, and Kilham, J A Bile Pigments and Bile Acids of the Blood in Jaundice, *Am J M Sc* 175 103 121, 1928
- 15 Byrom, F B, and Kay, H D Blood Phosphorus in Health and Disease III, A The Alleged Value of Blood Phosphorus Determinations in Suspected Malignant Disease B The Phosphorus Distribution in Anaemia, Polycythaemia and Leukaemia, *Brit J Exper Path* 9 72 81, 1928
- 16 Fahræus, R The Suspension Stability of the Blood, *Acta Med Scandinav* 55 1 228, 1921
- 17 Pinner, M, Knowlton, K, and Kelly, R G Sedimentation Rate of Erythrocytes, Its Relation to Fibrin Value and Cholesterol Content and Its Application in Tuberculosis, *Arch Path* 5 810 827, 1928
- 18 Gram, H C On the Causes of the Variations in the Sedimentation of the Corpuscles and the Formation of the Crusta Phlogistica ("size," buffy coat) on the blood, *Arch Int Med* 28 312 330, 1921
- 19 Frisch, A, and Starlinger, W Ueber die klinische Verwertung der Senkungsgeschwindigkeit der Erythrocyten bei der Lungentuberkulose, *Med Klinik* 17 1147, 1921
- 20 Lohr, W Die Senkungsgeschwindigkeit der roten Blutkörperchen als diagnostisches Hilfsmittel bei chirurgischen Erkrankungen *Mitt a d Grenzgeb d Med u Chir* 34 229, 1927
- 21 Kóvacs, K Die Wert der Senkungsreaktion der roten Blutkörperchen bei inneren Erkrankungen, *Deutsche med Wchnschr* 49 785 786, 1923
- 22 Rubin, E H Sedimentation Reaction in Cancer, *Am J M Sc* 174 680 690, 1927
- 23 Geronne, A Zur Pathogenese einiger Formen des Ikterus, *Klin Wchnschr* 1 828 832, 1922
- 24 Eppinger, H In Kraus and Brugsch Spezielle Pathologie und Therapie innerer Krankheiten Berlin. Urban und Schwarzenberg Vol 6, pt 2, p 200, 1923
- 25 Klemperer, P, Kilham, J A, and Heyd, C G Pathology of "Icterus Catarrhalis," *Arch Path* 2 631 652, 1926
- 26 Vorschütz, J Verschiedene Hamagglutinationsbilder bei Ikterusfällen und ihre Bedeutung, *Arch f Exper Path u Pharmacol* 95 235 237, 1922
- 27 Klopstock, E Catarrhal and Syphilitic Jaundice, *Med Klin* 19 1156 1157, 1923

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## LABORATORY TECHNIC

### IODINE CONTENT OF FOODS Estimation of Iodins in Foodstuffs and Body Fluids

Leitch, I and Henderson, J M Biochem J London 22 1003, 1926

Reagents and apparatus required

- 1 Potassium hydroxide sticks iodine free
- 2 Alcohol for extraction 95 cc absoluts alcohol, 5 cc distilled water
- 3 Methyl orange 0.05 per cent watery solution
- 4 Sulphuric acid approximately 2 N
- 5 Bromine water to be prepared as required
- 6 Sodium thiosulphate approximately N/500 This solution should be made up periodically from N/10 stock solution N/500 thiosulphate does not keep well and must be standardized daily by means of potassium biiodate as described by Keodall (1920)
- 7 Solution of potassium iodide made up just before the final titration A small crystal is dissolved in about 20 cc of water
- 8 Starch solution to 50 cc distilled water add a pinch of soluble starch Boil for one to two minutes and cool A fresh solution should be prepared every third day
- 9 Small chips of pumice stone about the size of rice grains These chips after removing dust through a fine sieve are boiled in dilute nitric acid washed with distilled water, dried, and then strongly beaten in the crucible furnace After the estimation they are recovered From time to time they are retreated and used over again
- 10 Davis combined crucible furnaces with Teclin burners Vitreosil basins for the sand are most suitable
- 11 Nickel crucibles (6 cm and 5 cm in diameter) Nickel stirring rods
- 12 Flasks (50 cc capacity)
- 13 A serum pipette 0.1 cc capacity graduated in thousandths of a cc

### PROCEDURE

**Ashing**—A suitable quantity of the substance to be analyzed is measured into a nickel crucible (6 cm diam) and 1 gm of potassium hydroxide stick added, dissolved in water in the case of dry substances The contents of the crucible are stirred and then heated gently over a Bunsen burner until all bubbling ceases and thereafter more strongly until no more fumes come off Heating is continued on the furnace to a dark gray ash The crucible must not be allowed to glow When cool the ash is moistened with distilled water and carefully heated over a low flame until dry The crucible is then returned to the crucible furnace and heated until no further change appears to be taking place (say ten minutes) After cooling, about 5 cc of distilled water are added and the contents of the crucible filtered through a No 40 Whatman ash free filter paper (In all pouring operations it is necessary to vaseline the lip of the crucible with iodine free vaselin) The filter paper is washed three times with about 5 cc of water The filtrate collected in a small beaker, is set aside It should be clear or at most, only very faintly yellow

The filter paper with its charred contents is returned to the nickel crucible dried and then heated in the crucible furnace until a clean ash is obtained The crucible is cooled, the filtrate returned to it and evaporated to dryness This operation requires the greatest caution towards its completion since spitting and cracking may cause loss The crucible is again heated in the furnace for a minute or so then cooled and about 3 cc of water are added This thick solution is gently evaporated over a low Bunsen flame until a skin begins to form on the surface Then the contents after cooling are ready for extraction

**Extraction**—The ash is extracted three times with 3 cc of the alcohol solution, the extract being filtered through a No 40 Whatman paper into a 5 cm crucible The ash



should form a smooth paste. If it is gritty one or two drops of water should be added, but excess should be avoided. After the third extraction the paste is poured on to the filter and the last of the alcohol allowed to drain. The filter paper is rejected, the funnel washed with a little distilled water and the filtrate evaporated to dryness on the water bath.

The thin film of salt on the bottom of the crucible should be practically free from carbon. To get rid of any small particles which may be present, the bottom of the crucible is glowd gently for a few seconds, the salt film being just allowed to melt.

When the crucible has cooled the contents are dissolved in distilled water, and transferred to a 50 cc flask.

**Titration**—Two drops of the methyl orange solution are added. The solution is rendered neutral by the addition, drop by drop, of 2N sulphuric acid. One drop is added after the neutral point. In all about three drops are generally required. Thereafter a few drops of bromine water are added. The red color is immediately discharged. About 1 cc of bromine water should be added in excess of this, until the solution is distinctly yellow.

Three or four pieces of pumice stone are added and the solution is boiled over a medium flame, until the volume is reduced to between 1 and 2 cc, and then cooled. Two drops of the potassium iodide solution and two drops of the starch indicator are added and the titration carried out with N/500 thiosulphate from the 0.1 cc pipette. By holding the flask at an angle it is possible by tapping the point of the pipette to run in as little as 0.001 cc at a time. If due precautions have been taken, the end point is perfectly definite and after a little practice may be determined to about 0.002 cc.

Calculation

Titration figure  $\times$  iodine equivalent of thiosulphate

9

gives the amount of iodine in the sample

**PROTEIN DETERMINATION** Colorimetric Determination of Proteins in Plasma, Cerebrospinal Fluid, and Urine, Wu, H., and Ling, S. Chinese J Physiol 1 No 2, 161, 1927

Standard solution	Tyrosine 50 gm dissolved in 250 cc N/0.1 HCl	Phenol Reagent
	$\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$	100 gm
	$\text{Na MoO}_4 \cdot 2\text{H}_2\text{O}$	25 gm
	Water	700 cc

Dissolve and add 50 cc of 85 per cent  $\text{H}_3\text{PO}_4$  and 100 cc concentrated HCl. Boil with condenser eight hours and dilute to one liter.

#### DETERMINATION OF PROTEINS IN PLASMA

**Determination of Fibrin** To 1 cc of the plasma, from blood containing 0.2 to 0.6 per cent potassium oxalate, add 28 cc of 0.8 per cent NaCl solution and 1 cc of 2.5 per cent  $\text{CaCl}_2$  solution. Mix and allow to stand undisturbed until a solid clot has formed. Break up the jelly by shaking slightly and transfer it to a dry filter. While filtering insert into the jelly a slender glass rod with a pointed end and whirl gently. All the fibrin will stick to the rod. Slip the fibrin off the rod, and press it between dry filter paper to remove the adhering liquid. Transfer it to a 15 cc centrifuge tube, add 4 cc of 1 per cent sodium hydroxide. Place the tube in a boiling water bath and stir with a slender glass rod until the fibrin lump has completely disintegrated leaving the calcium oxalate in suspension. Add 10 cc of water, mix, and centrifuge. Transfer the supernatant liquid to a 25 cc volumetric flask or graduated tube. Cool under the tap. Add 1 cc of 5 per cent  $\text{H}_2\text{SO}_4$ , 0.5 cc of phenol reagent, and dilute to about 20 cc. Add 1 cc of 10 per cent NaOH solution. Mix by gentle rotation, make up to volume.

The standard is prepared as follows. Measure 1 cc of the standard tyrosine solution into a 25 cc volumetric flask or graduated tube, add 0.5 cc of phenol reagent, dilute to about 20 cc and finally add 1 cc of 10 per cent NaOH solution. Make up to volume and mix. The standard should, of course, be prepared at the same time as the unknown. Let stand for one hour before making the color comparison.

If any fibrin fails to stick to the rod it should be picked up with the tip of the rod. If the amount of the fibrin in the plasma is very high, say 0.8 per cent or more the fibrin jelly will not shrink readily. In such a case it is necessary to use less of the plasma for the determination.

**Calculation** If the standard is set at 20 and the reading of the unknown is  $R$ , then the amount of the apparent tyrosine determined is  $\frac{20}{R} \times 0.2$  mg. Since 1 mg of tyrosine = 11.3 mg of fibrin, the amount of fibrin in 1 cc of plasma is  $\frac{20}{R} \times 0.2 \times 11.3$  mg or the percentage of fibrin =  $\frac{20}{R} \times 0.226$ .

**Determination of Albumin** To 1 cc of plasma add 4 cc of  $H_2O$  with 5 cc of saturated ammonium sulphate solution, or 9 cc of saturated magnesium sulphate solution and 0.3 gm of anhydrous magnesium sulphate. Mix and allow to stand for thirty minutes. Filter. Measure 1 cc of the filtrate into a 15 cc centrifuge tube. Add about 12 cc of  $H_2O$ , 1 cc of 10 per cent sodium tungstate solution and then 1 cc of  $2/3$  N sulphuric acid. It is advisable to add one or two more drops of acid. Stir thoroughly with a slender glass rod and centrifuge. Carefully decant the supernatant liquid as completely as possible. The volume of the wet precipitate usually amounts to about 0.5 cc. If it is much smaller than 0.5 cc indicating low albumin, measure another cc of albumin filtrate into the same tube, dilute with water and proceed as before. Add to the precipitate in the centrifuge tube 1 cc of sodium tungstate solution. Stir until the precipitate has dissolved, dilute with 13 cc of  $H_2O$ , and add 1 cc of  $H_2SO_4$ . Stir again, centrifuge and decant the supernatant fluid. This second precipitation is intended to remove the calcium and ammonium or magnesium so nearly completely that they cannot possibly interfere with the subsequent color reaction although experience has shown that a single precipitation usually suffices. Add to the precipitate in the tube 10 cc of  $H_2O$  and 1 or 2 drops (but no more) of 20 per cent  $Na_2CO_3$ . Stir until the precipitate has dissolved. Transfer the resulting solution to a 25 cc volumetric flask or graduated tube. Rinse the centrifuge tube twice with 3 cc of  $H_2O$ . Add 0.5 cc of phenol reagent and 1 cc of 10 per cent  $NaOH$  solution. Mix by gentle rotation. Make up to volume and mix. Prepare a standard as in the fibrin determination and read the color after one hour.

**Determination of Albumin and Globulin** Measure 2 cc of the filtrate in the fibrin determination into a 15 cc centrifuge tube and proceed exactly as in the determination of albumin.

**Calculation** In the calculation it is to be noted that the solution used for the albumin determination is plasma diluted 1 to 10, while that used for the determination of albumin and globulin is plasma diluted 1 to 30. If 1 cc of the albumin solution is used for the former determination and 2 cc of the serum solution are used for the latter determination and the colorimeter readings are  $R$  and  $R_1$  respectively, the standard being set at 20 then the total apparent tyrosine in 1 cc of serum =  $15 \times \frac{20}{R_1} \times 0.2$  mg the apparent tyrosine of albumin in 1 cc of serum =  $10 \times \frac{20}{R} \times 0.2$  mg and the apparent tyrosine of globulin in 1 cc of serum is

$$(15 \times \frac{20}{R_1} \times 0.2) - (10 \times \frac{20}{R} \times 0.2) = \frac{60}{R_1} - \frac{40}{R} \text{ mg}$$

Since 1 mg of tyrosine = 15.8 mg of globulin = 21.4 mg of albumin of human serum

$$\text{per cent of globulin} = \frac{(\frac{60}{R_1} - \frac{40}{R}) \times 15.8}{1000} \times 100 = (\frac{6}{R_1} - \frac{4}{R}) \times 15.8$$

$$\text{per cent of albumin} = \frac{(10 \times \frac{20}{R} \times 0.2) \times 21.4}{1000} \times 100 = \frac{20}{R} \times 4.28$$

## DETERMINATION OF PROTEIN IN URINE

Transfer to a 15 cc centrifuge tube 15 cc of filtered urine and dilute with water to about 10 cc. Add 2 cc of 10 per cent sodium tungstate and 2 cc of 2/3 N sulphuric acid. The mixture is stirred thoroughly with a slender glass rod and centrifuged. In case the supernatant liquid is milky in appearance which occurs only in alkaline urine, add a few more drops of 2/3 N sulphuric acid and the mixture is stirred and centrifuged again. The water clear supernatant liquid is carefully decanted. To the precipitate in the centrifuge tube add 1 cc of sodium tungstate and stir until it has completely dissolved. Dilute with about 10 cc of water and add 1 cc of 2/3 N sulphuric acid. The mixture is again stirred, centrifuged, and the supernatant liquid decanted. This procedure is repeated once more in order to remove completely those nonprotein substances which react also with the phenol reagent. Finally, the precipitate is suspended in 10 cc of water and dissolved with one or two drops of 20 per cent sodium carbonate solution. If the volume of the wet precipitate amounts to about 0.4 cc the whole solution is transferred to a 25 cc volumetric flask or graduated tube and the centrifuge tube rinsed twice with 3 cc of water. If the volume of the precipitate is much smaller than 0.4 cc the determination should be repeated with more urine. If it is more than 0.5 cc the final solution is made up to 10 cc and an aliquot portion is used for the determination. The rest of the procedure is the same as described above.

**Calculation** If the standard is set at 20 and the reading of unknown is R, then the apparent tyrosine determined is  $\frac{20}{R} \times 0.2$  mg.

Since the protein in the urine is largely albumin, 1 mg tyrosine may be taken as equivalent to 20 mg urinary protein. If the amount of urine used is n cc the amount of protein in 100 cc urine is  $\frac{20}{R} \times 0.2 \times 20 \times \frac{100}{n}$  mg =  $\frac{20}{R} \times \frac{400}{n}$  mg or the percentage of protein in urine is  $\frac{20}{R} \times \frac{4}{10n}$ .

## DETERMINATION OF PROTEIN IN CEREBROSPINAL FLUID

Transfer to 15 cc centrifuge tube 2 cc of spinal fluid. Add 3 cc H<sub>2</sub>O followed by 1 cc each of 10 per cent sodium tungstate and 2/3 N sulphuric acid. The mixture is stirred and centrifuged. Decant the supernatant liquid. Add to the precipitate 1 drop of 10 per cent Na<sub>2</sub>CO<sub>3</sub> and H<sub>2</sub>O to the 5 cc mark. Stir until the precipitate has dissolved. Add 0.2 cc of phenol reagent and 1 cc of 5 per cent NaOH. The content of the tube is mixed by inversion. After one hour the color is compared in a usual manner against a standard prepared at the same time as the unknown.

Two standards are prepared as follows. Into two similar test tubes marked Standard 1 and Standard 2 are measured 0.2 cc and 0.4 cc of the standard tyrosine solution, and 4.8 cc and 4.6 cc H<sub>2</sub>O respectively. Then add to each 0.2 cc of phenol reagent and 1 cc of 5 per cent sodium hydroxide. The solutions are mixed by inverting the tubes. The standard which is closer, by inspection, to the unknown is used for the comparison.

**Calculation** We have not been able to collect enough cerebrospinal fluid for the determination of the tyrosine equivalent by the Kjeldahl method. For clinical purposes the tyrosine equivalent of the protein in spinal fluid may be taken as 18, the average of the values for serum albumin and globulin. If Standard 1 is used and set at 15, then  $\frac{15}{R} \times 0.04 \times \frac{100}{2} \times 18$  = mg of protein per 100 cc of spinal fluid. With Standard 2 set

at 15,  $\frac{15}{R} \times 0.08 \times \frac{100}{2} \times 18$  = mg of protein per 100 cc of spinal fluid.

## DETERMINATION OF SOLUBLE PROTEINS

The colorimetric method is especially useful for comparing relative concentrations of protein solutions. If the protein solutions contain no interfering substances, suitable volumes are measured into 25 cc graduated tubes and the color developed directly. If the solutions

contain interfering substances, the protein is first precipitated with suitable amounts of sodium tungstate and sulphuric acid

If it is desired to calculate the absolute concentration of the protein, it is of course necessary to determine beforehand the tyrosine equivalent of the protein in question by parallel tyrosine and nitrogen determinations

**CEREBROSPINAL FLUID The Fuchsin Sublimate Reaction (Takata Ara) Kafka.**  
München med Wchnschr 73 1830, 1926

The practical execution of Takata and Ara's new cerebrospinal fluid reaction consists in the mixing and shaking thoroughly of 1 cc of cerebrospinal fluid with a drop (0.033 cc) of a 1 per cent sodium carbonate solution and 0.3 cc of a freshly prepared mixture of equal parts of a 0.5 per cent sublimate and a 0.2 per cent fuchsin solution. The normal cerebrospinal fluid shows a bluish purple color and remains clear.

In case of a syphilitic nervous system which has already changed the protein fraction of the cerebrospinal fluid, a flocculation with bluish purple sediment appears, above which is a transparent liquid.

In case of a non-syphilitic meningitis with a protein quotient similar to the protein content of the serum a rose discoloration without deposit is observed.

Four degrees of flocculation can be distinguished in the syphilitic type of the reaction, the intensity of which is dependent upon the length of the time until the sediment appears. An immediate total flocculation indicates the highest degree while 3 slighter ones are observed after fifteen and thirty minutes and twenty four hours.

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### Practical Lectures\*

THIS work covers such a wide variety of subjects that it does not lend itself easily to review. The Medical Society of the County of Kings, Brooklyn, N. Y., instituted what might be termed a postgraduate course in which authorities on a wide variety of the aspects of medicine and surgery were invited to deliver lectures to the Society, lectures which are primarily practical in that they gave forth information which may be applied with advantage in the routine practice of medicine. The present volume is the second of a series being published by this Society. It should be of great value to the general practitioner who will find between the covers a quite comprehensive postgraduate medical instruction.

### The Medicine Man†

THOSE who enjoy medical biographies, and every physician should, will look forward to reading Dr. E. T. Dudley's autobiography. Here is the story of a man who actually did work his own way from even his primary education, on and upward to that soul-satisfying point where he had received the highest honors in his branch of the profession, had retired as president of a great medical school and had received the recognition of the medical world for his scientific contributions.

Dr. Dudley came from sturdy New England stock, started to earn his own way at the tender age of thirteen as a clerk in an apothecary shop, worked his way through Dartmouth and through Yale, graduated from Long Island Medical College, procured for himself the best possible training in gynecology under the associates of J. Marion Sims and then settled in Chicago where he grew with the city and where Northwestern University Medical School grew with him.

Most of us do not settle down to enjoy medical biography until we have reached that point where we find sufficient leisure in our busy day for this type of relaxation. A medical man at any age will enjoy this autobiography but its greatest inspiration will be to the young man of little or no financial resources who is contemplating the field of medicine as a lifetime study. Here he will find all of the encouragement he will need, but unfortunately it is he who will probably be least likely to discover the book.

An autobiography to be really thrilling must make somewhat of a hero out of the principal character. The great risk therefore becomes the appearance of too great egotism. Edward Bok wrote his autobiography in the third person so as to avoid the large I but his contribution is far more egotistical than that of Dr. Dudley.

His record of his adventures is simply recorded and with a wealth of dry and subtle humor. His deductions from his experiences display the mental workings of a true philosopher. His fund of anecdote is charming.

We suspect that the unburnt tint of Dr. Dudley's hair has been quite a factor in his usually getting what he set out after. The red-headed boy is usually meat for his companions' buffoonery and in self-protection he develops a self-reliance and unshakable determination. Some day we hope to read a scientific dissertation on the psychology of the red-topped youngster.

Dr. Dudley takes us through the early days of medical apprenticeship, the two-year medical school in which the second-year lectures repeat the first-year, the days of septic surgery, of antiseptic surgery and finally of aseptic surgery. We notice with surprise and with some regret that he has practically nothing to say of the development of medical education in the great Chicago school with which he was associated.

There is quite a scattering of typographic errors which, however, in no way detracts from the pleasure of the book.

*Practical Lectures on the Specialties of Medicine and Surgery.* Delivered under the Auspices of The Medical Society of the County of Kings, Brooklyn, New York. (Second Series 194-108.) With one hundred and ten illustrations. Cloth. Pages 590. Paul B. Hoeber, Inc. New York, 1927.

*The Medicine Man: Being the Memoirs of Fifty Years of Medical Progress.* By E. C. Dudley, M.D., LL.D., Ex-President American Gynecological Society, President Emeritus of Northwestern University Medical School. J. H. Sears & Co. Inc. New York, 1927.

### *Sulphur Metabolism\**

THE late Dr Kahn and Dr Goodridge, while studying the biochemistry of sulphur compounds found that the task of covering the literature of sulphur compounds in plant and animal metabolism was enormous. They therefore decided that to aid others who might sometimes carry studies along similar lines it would be well to prepare an exhaustive résumé of the entire literature on the subject. Thus they have done in a volume of 831 pages.

They have not been content with merely recording the bibliography but have reported in detail those observations in the literature which to them appeared of primary importance together with a wealth of tabulation and have classified the literature and arranged it in coordinated sequence.

The great value of the work will, as the authors have indicated, be as a reference organ for the student of the metabolism of sulphur.

### *The Elements of General Zoology†*

IN THE profusion of textbooks here is one that is truly different. The difference consists in that while most textbooks on elementary zoology stress the study of structure Dr Dakin's text correlates the structural studies with functional studies. Much more space is given to the latter than to the former. Indeed, we might designate this volume as a work on comparative anatomy, physiology, and embryology of those forms of life which are usually studied in the zoologic laboratory.

### *Textbook of Medicine‡*

A SINGLE volume of 1500 pages designed primarily as a student text and as a quick reference volume. There are 130 contributors to the volume every one of whom is an outstanding authority. Indeed, one marvels in looking over the list of contributors that the editor has succeeded in collecting such a galaxy of names.

The discussion of the individual diseases is necessarily brief but is at the same time concise and pointed. We anticipate that this textbook will come into quite general use.

### *The Normal Chest§*

THE anatomy, physiology, embryology, roentgen, and physical aspects of the normal chest. The undergraduate medical student usually studies anatomy one year, physiology the next and takes up clinical studies after he has forgotten much of the former. The volume under consideration correlates all of these studies of the thorax into one systematized review. There is no discussion of pathology. The volume should be of value not only to undergraduate students but to all who wish from time to time to review concisely their knowledge on the subject of the chest.

\*Sulphur Metabolism. A Review of the Literature. By Max Kahn, M.A., M.D., Ph.D., Associate in Biological Chemistry, School of Medicine, Columbia University; Visiting Physician in Diseases of Metabolism and Director of Laboratories, Beth Israel Hospital; Visiting Physician, United-Israel-Zion Hospital and Frederic G. Goodridge, M.D., Ph.D., Associate in Biological Chemistry, School of Medicine, Columbia University. Cloth. Pages 831. Lea & Febiger, Philadelphia and New York, 1926.

†The Elements of General Zoology. A Guide to the Study of Animal Biology Correlating Function and Structure with Notes on Practical Exercises. By William J. Dakin, D.Sc., F.Z.S., Professor Zoology in the University of Liverpool. Cloth. Illustrated. Pages 496. Oxford University Press, American Branch, New York, 1927.

‡A Textbook of Medicine. By 130 American Authors. Edited by Russell L. Cecil, M.D., Assistant Professor of Clinical Medicine, Cornell University Medical School, New York. Octavo of 1500 pages illustrated. Cloth. W. B. Saunders Company, Philadelphia and London, 1927.

§The Normal Chest of the Adult and the Child. Including Applied Anatomy, Applied Physiology, X-Ray and Physical Findings. By J. A. Myers, Associate Professor of Preventive Medicine, Medical and Graduate Schools, University of Minnesota; Medical Director, Lymanhurst School for Tuberculous Children, Minneapolis, Minn. 143 illustrations, 191 references. Cloth. Pp. 400. The Williams & Wilkins Company, Baltimore, Md., 1927.

We would mention particularly the discussion of the lymphatic supply of the thorax the discussion of routine systematic examination, innervation of the chest, the developmental anatomy of the chest and the acoustics of percussion and auscultation. The illustrations are remarkably clear and descriptive adding greatly to the value of the work

### *Tonic Hardening of the Colon\**

**G**ASTROINTESTINAL function, or rather dysfunction is receiving more and more attention. Possibly the day is not far distant when such diagnoses as dyspepsia nervous indigestion, gastric neurosis and intestinal indigestion will not be needed to cover up ignorance.

Wilson's monograph upon a common colonic condition is an important stepping stone toward a better understanding of the nervous dyspeptic. It throws new light upon spastic constipation. The work is divided into three parts: symptomatology, clinical section, physiologic considerations.

The general practitioner and gastroenterologist alike will spend an interesting as well as profitable hour in the perusal of this monograph.

### *Pharmaceutical Bacteriology†*

**T**HIS is a textbook on bacteriology intended for the use of pharmacists.

It is questionable whether the pharmacist should be prepared to conduct a clinical laboratory and equally questionable whether this book will enable him to do so.

### *Brain and Heart‡*

**T**HE title of this small work is regrettably misleading and gives no conception of the material between the covers. The work consists essentially of a series of philosophic deductions based in part upon Fano's extensive experimental studies in physiology. The first portion might be aptly termed a physiologist's conception of the nature of living matter. The second might well be termed personal ideas on the origin of life and the development of species. Fano is inclined to doubt the theory that life ever developed out of an azoic period. Rather he says life has always existed and inorganic matter has developed as a result of the activities of life. He accepts neither Darwinism nor the Lamarckian philosophy.

### *Chronic Rheumatic Diseases Their Diagnosis and Treatment§*

**T**HERE is a tale of a man who rushed into a busy practitioner's office and who impatient of delay demanded a prescription for rheumatism. He was given the prescription and as he was hurrying down the street was called back by the physician who said: "If the medicine helps you be sure to let me know for I have rheumatism too."

The present treatment of chronic arthritis almost entirely palliative is on the whole so unsatisfactory as to be a source of regretful embarrassment to the physician.

*Tonic Hardening of the Colon.* By T. Stacy Wilson, M.D., B.Sc. (Edin.), F.R.C.P. (Lond.). Cloth. Pages 70. Oxford University Press American Branch New York, 1927.

*Pharmaceutical Bacteriology.* By Albert Schneider, M.D., Professor of Pharmacognosy, University of Nebraska. Second Edition. Cloth. 9 illustrations. 411 pages. P. Blakiston's Son and Co., Philadelphia.

*Brain and Heart. Lectures on Physiology.* By Giulio Fano of the Royal University of Rome. Translated by Helen Ingleyby with a foreword by Prof. E. H. Starling, M.D., M.R.C.P. D.Sc., F.R.S. University College London. Cloth. Pages 14. Oxford University Press American Branch New York, 1928.

*Chronic Rheumatic Diseases Their Diagnosis and Treatment.* By F. G. Thomson, M.A., Cantab., M.D., F.R.C.P. (Lond.), Physician to the Royal United Hospital Bath Consulting Physician to the Royal Mineral Water Hospital Bath, etc. and R. G. Gordon, M.D., D.Sc., M.R.C.P. (Edin.), Physician to the Royal Mineral Water Hospital Bath, Physician to the Children's Orthopedic Hospital Bath, Assistant Physician to the Royal United Hospital Bath. Cloth. Pages 202. Oxford University Press American Branch New York, 1928.



Dr Crowe on the other hand is most optimistic and claims with his methods to have achieved excellent results. In the present monograph he presents his methods and results with a promise to give in a succeeding contribution more detailed presentation of his experimental work and laboratory methods.

The author first recognizes various types of arthritis and stresses the importance of knowing in so far as possible with what type the physician is dealing. He recognizes the beneficial effect of supportive and palliative therapy as is customarily applied but in his specific work he attempts to build up the individual's general resistance to invading bacteria. His treatment is, briefly, vaccine therapy, but vaccine therapy very carefully controlled. The two microorganisms which in his experience are mainly responsible are a *Staphylococcus albus* designated by him *micrococcus deformans* and a *streptococcus*. These are usually natural inhabitants of the intestinal tract and may be present in infectious foci.

The author recommends the use of a stock mixed vaccine of these organisms isolated from the various foci but particularly from the intestinal tract but emphasizes that his excellent results have been due to his carefully controlled dosage and administration. He uses small doses rather than large ones. There is so much detail in the supervision of administration that one desiring to follow the author's methods must perforce carefully study the monograph.

### *Pathological Physiology of Internal Diseases\**

A SAD and regrettable consequence of passage from this life, among medical writers at least, is that their works which previously may even have been standard reference literature gradually find their way to the dusty shelves to be delved into only by the student of past history. This is especially regrettable in the case of Hewlett's *Functional Pathology* for it is a contribution of utmost value and we owe a debt of gratitude to his colleagues who, on the basis of Hewlett's revisionary notes and their own knowledge and experience have brought his functional pathology up to the minute and dedicated it as a memorial volume.

The classifications and arrangements are practically unchanged for indeed they could scarcely have been improved upon.

We believe we are safe in saying that Hewlett's *Functional Pathology* either in its present form or as it originally appeared as a volume of *Monographie Medicale* will be found in as many private medical libraries as very nearly any other one medical treatise.

### *Lobar Pneumonia†*

A COMPREHENSIVE presentation of the evolution and course of lobar pneumonia is recognized and studied by the roentgenologist. The volume is rather small, concise and at the same time quite inclusive. In most phases of the discussion the bacteriologic findings receive little attention. In the discussion of lobar pneumonia, we would have liked especially to have known the bacteriologic findings.

The author establishes convincingly his case to the effect that a ray examination is of greatest assistance in the early recognition of pneumonia and especially in the early discovery of complications. We are rather disappointed not to see some mention based on his wide roentgen experience of abortive types of pneumonia, the so called three day pneumonia. He leaves the question uncertain as to its existence.

\**Pathological Physiology of Internal Diseases. Functional Pathology.* By Albion Walter Hewlett, M.D., B.S. Formerly Professor of Medicine, Stanford Medical School; Professor of Internal Medicine and Director of Clinical Laboratory, University of Michigan. Revised in Memoriam by his Colleagues. Under the Editorial Supervision of George DeForest Barnett. With one hundred and sixty-four illustrations in text. Cloth. 787 pages. D. Appleton & Company, New York.

†*Lobar Pneumonia. A Roentgenological Study.* (A Correlation of Roentgen-Ray Findings with Clinical and Pathological Manifestations). By L. R. Sante, M.D., F.A.C.R., F.A.C.P., Associate Professor Radiology, St. Louis University Medical School; Radiologist to the University Group of Hospitals (St. Mary's Hospital and Infirmary); Chief City Radiologist to St. Louis City Hospitals; Consultant Radiologist, Koch Hospital for Tuberculosis, St. Louis Training School, etc. Illustrated. Cloth. Pp. 137. Paul B. Hoeber, Inc., New York, 1923.

### *Bacteriology and Surgery of Chronic Arthritis and Rheumatism\**

IN CROWE'S first volume which has been reviewed in these columns he promised a second in which the details of technique would be discussed in greater detail. We have before us this second volume which we suspect has taken on a slight different form from the author's original intention, the new form developing naturally with the evolution of his discussion. A large first section continues to deal with the etiology of arthritis. This is followed by a critical study of his own therapeutic results, a rather brief discussion of bacteriologic technique (we had anticipated rather more), and a classification of the streptococci which he has obtained from arthritic cases. A chapter on physical treatment is contributed by Herbert Frankling.

Crowe's mode of attack on arthritis is from the bacteriologic viewpoint and his results arrest attention. The streptococcus is quite generally conceded to be a factor in arthritis but *Staphylococcus albus* has not been as positively incriminated by other workers.

This volume like its predecessor should be in the hands of all who undertake a serious study of arthritis.

### *Elements of Physiology for Students of Medicine and Advanced Biology†*

HERE is an interesting work. We prick up our ears when we read the preface and then turn to the text with some apprehension for we have often read in prefaces of new modes of attack only to be disappointed on finding the same old routine presentation in the text.

In the preface the authors announce that the subject is presented by the development of two keynote principles. The first is that living protoplasm is a system of molecules and ions, hence, understanding of its structure and function is to be sought by study of those physical, physicochemical and chemical laws which govern the interrelations of molecules and ions. The first section therefore contains an account of the nature and capacities of protoplasm as developed primarily from a study of individual cells and their environmental reactions. The second key principle is that every protoplasmic cell is inherently a self-sustaining system and provided its environment is right it should continue to live and function almost indefinitely.

In other words after reading the preface we hope to find a consecutive development of the principles of human physiology, along pure biologic lines beginning with the physiology of the individual cells and their reactions to environmental alterations and their developmental changes consequent on functional specialization.

To our surprise and delight we find that these authors have done what they announced they would do. The result is a quite comprehensive treatise on human physiology which reads more like a story book than textbook and in which the plot is gradually and logically developed.

### *Bolles Lee's Microtometist's Vade Mecum‡*

THIS volume is so well known to the tissue pathologist that its ninth edition requires no introduction. It is sufficient to say that the present edition of Lee's monumental work is edited by Gatenby of Dublin and Cowdry of New York with the collaboration of eleven men preeminent in their field. We might describe the work as the standard reference encyclopedia for tissue pathologists.

\**Bacteriology and Surgery of Chronic Arthritis and Rheumatism With End Results of Treatment.* By Warren Crowe, D.M. B.Ch. (Oxon), M.R.C.S., L.R.C.P. The Chapter on Surgical Treatment by Herbert Frankling, C.B.E., M.R.C.S. (Eng.) Cloth. Illustrated. Pp. 187. Oxford University Press American Branch New York, 1927.

†*Elements of Physiology for Students of Medicine and Advanced Biology.* By Earnest G. Martin, Ph.D., Professor of Physiology at Stanford University and Frank W. Weymouth, Ph.D., Associate Professor of Physiology in Stanford University. Cloth. Pp. 784. Lea & Febiger, Philadelphia, 1928.

‡*Bolles Lee's Microtometist's Vade-Mecum. A Handbook of The Methods of Microscopic Anatomy.* Ninth Edition Edited by J. Bronte Gatenby and E. V. Cowdry. With Nine 11 Illustrations. Cloth. Pp. 710. P. Blakiston's Son & Company, Philadelphia, Pa. 1928.

### *Lectures on Medicine and Surgery\**

VOLUMES containing collections of addresses by eminent men on very practical aspects of the practice of medicine and surgery are gaining deserved recognition. As a consequence several organizations are entering this field. One of the latest is the New York Academy of Medicine. This Academy has a wealth of good material to draw upon and it has selected wisely in its contributions to its first series of lectures on medicine and surgery.

A very readable lecture on the Surgical Aspects of Medical Conditions brings to mind that another contribution of equal or even greater value would be one on the Medical Aspects of Surgical Conditions. Too often when the postoperative case goes bad the surgeon rather glibly tells the anxious relatives that the kidneys have played out or that the heart is failing when there is no clinical justification for such a diagnosis.

Out our way we speak of two types of digitalis dosage, the medical dose and the surgical dose. The surgeon too often when the pulse gets a little rapid gives a single hypodermic injection of some variant of digitalis and settles back content with the self assurance that he has digitalized his patient. It reminds one of the man who attempts to tickle the elephant with a straw.

George M. MacKee in a lecture on The Cutaneous Manifestations of Syphilis produces one of the best series of illustrative photographs that I have had occasion to see. They remind us that the badly disfiguring and ulcerative lesions of tertiary syphilis are not yet entirely things of the past.

Dr. James Alexander Miller contributes a lecture on Climate in Tuberculosis. He discusses climatic factors such as temperature, humidity, wind, dust, storm, character of the soil, and vegetation and configuration of the country. His conclusions are worthy of repetition, that while many cases can be satisfactorily treated in or near their home environment, others actually do better in other climates and the climate for one patient is not necessarily the appropriate one for another. Many factors must be considered in the selection of the proper climate for a tuberculous individual.

Some will disagree with certain of the statements by Louis K. Neff on Useful Drugs in Clinical Practice. But this is true in nearly all such discussions for we try to generalize on our experience with individual cases, failing to realize that each is different and must be treated on its own merits.

David Riesman contributes an excellent discussion of the Treatment of Pneumonia. While we have no proved specific for pneumonia, so much has been written on fundamental principles in this disease that it would seem superfluous to reiterate them. But every consultant knows from his own experience that the average general practitioner is still treating pneumonia as it was treated thirty years ago with unjustified stimulation and improper use of digitalis. Dr. Riesman's article is deserving of a wide audience.

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### *Clinical Case-Taking†*

ONE can recognize distinct advantages in Dr. Herrmann's decision to publish this small manual as a separate and supplementary volume to his *Methods in Medicine*, for this is a work which the beginner in physical examination will want to have available even at the bedside and it should therefore be in a handy compact form.

Nonessentials appear to have been well eliminated and yet the details of history taking and physical examination are described well enough so that the work is quite complete for the purposes for which it is intended.

The graduate who has dropped out of the habit of painstaking examination will derive great help from its use.

\*The New York Academy of Medicine Lectures on Medicine and Surgery. With Thirty-Nine Illustrations. Cloth. Pp. 319. Paul B. Hoeber, Inc., New York, 1928.

†Clinical Case-Taking. Supplement to *Methods in Medicine*. By George R. Herrmann, M.D., Ph.D., Assistant Professor of Medicine, Tulane University, New Orleans. Cloth. Pp. 90. The C. V. Mosby Company, St. Louis, Mo., 1927.

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Official Organ of the American Society of Clinical Pathologists and the  
American Association for the Study of Allergy

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## EDITORIALS

### A Neglected Field for Studies in Heredity

**M**ALIGNANT neoplastic diseases are apparently becoming more significant in biology and in the practice of medicine. Many valuable observations made in the past that have advanced the knowledge of malignant diseases in man have resulted from studies of malignant tumors as they are found in animals. More extensive observations and general interest in malignant tumors as they occur in animals may further aid in the solution of some of the problems in the investigation of cancer.

Most of the data concerning spontaneous malignant neoplasms have been obtained from the smaller laboratory animals, particularly mice. Obviously domestic animals that are used for food are usually killed before they have reached the age at which neoplastic changes usually occur.

In horses and dairy cattle, however, breeding lines are known for many generations. For example the thoroughbred line of horses was founded largely from the blood lines of three sires Godolphin, Bart, and Turl.

Darley Arabian These three horses were brought to England in 1724, 1689, and 1706, respectively The get of this breed are almost all on record and they include thousands of individuals and a great many generations of animals Godolphin Bart alone was the sire of more than 1200 colts The father of the American breed of trotting horses is generally conceded to be Hambletonian X This colt was foaled May 15, 1849, and his ancestors were known for five generations More than sixteen generations of horses trace their lineage back to this famous sire Intimate data regarding these animals are known, their track records, their offspring and their pedigree The breeding lines of certain dairy cattle go back a great many generations Milking records and breeding data are all recorded

All of this great mass of data, which is of value to a student of genetics, is available, but an important element of economic significance and importance in human affairs is missing that is, information regarding the kind and incidence of neoplastic diseases present in these animals Even if new-growths are rare it would seem that data on this fact are of importance Old pure bred cattle are usually butchered and there is no chance to follow them through the abattoir and determine the presence or absence of new-growths Horses, however, especially if they are of racing stock, usually live to old age and die of natural causes

Neeropsy of horses of racing stock by a competent veterinarian, and the microscopic examination of any tumors found, in consideration of the great number of colts from one sire and the large mass of data constantly accumulating, might prove a fruitful source of information for the study of the influence of genetic factors in neoplastic disorders

—H D C

### The Filtrable Form of the Tubercle Bacillus

EVERYONE who has searched sputum smears for hours without finding tubercle bacilli, while the same sputum readily causes tuberculosis in a guinea pig, must be anxious to believe in the possibility of the occurrence of another form of the tubercle bacillus than the common acid-fast one The question has arisen whether this other form may be the well-known Much's granules, or whether a real "fluidum contagium vivum" exists

Fontes<sup>1</sup> reported in 1910 that Berkefeld filtrates of tuberculous caseous material caused enlargement of the lymph nodes in guinea pigs He demonstrated acid-fast organisms in these lesions In 1922 and the following years Vaudremer<sup>2</sup> reported similar findings with filtrates from glycerin-free broth and potato water cultures He believed, as Fontes did, that the tuberculous virus passes the filter in the form of Much's granules These were formed abundantly in his cultures When the tubercle bacillus grew under the surface in potato water it developed a nonacid-fast mycelium and numerous granules Inoculation of the filtrate in guinea pigs caused enlargement of the lymph glands from which Vaudremer isolated tubercle bacilli of low virulence Valtis<sup>3</sup> inoculated filtrates of autolysed sputum and found acid-fast organisms in the lungs but there were no swollen lymph glands In another experiment

the filtered pus from a monkey node caused typical tuberculosis in a guinea pig. Ariong and Dufourt<sup>4</sup> obtained a filtrable form of low virulence from tuberculous organs which caused tuberculosis of the lymph glands, but they were unable to demonstrate the existence of a filtrable form in culture media. Nor could any of these authors confirm Vaudremer's findings of a nonacid fast mycelium. Several other French authors obtained similar results and in this country Mellon and Jost reported positive findings. On the other hand Cooper and Petroff<sup>5</sup> did not find any evidence of a filtrable form but observed acid fast granules in the lymph glands of 33 per cent of their controls. Montemartini,<sup>7</sup> Fessler,<sup>8</sup> Rossetti<sup>9</sup> and Ruys<sup>10</sup> had negative results.

Fontes used diatomaceous filters (Berkefeld), but most of the other workers used porcelain filters (Chamberland L2 and L3). According to the makers<sup>11</sup> the size of the pores in the Berkefeld filters varies from 4 to 12  $\mu$  according to the grade but Rosenthal<sup>12</sup> and Mudd<sup>13</sup> found that the effective diameter of the pores is much less, approximately 0.5  $\mu$ . The makers of the Chamberland filters state that the L2 filter permits small bacilli to pass and that the L3 filter retains even the diphtheria bacillus. It is evident that the mere fact that the tuberculous virus passes through these filters is not sufficient proof that we are dealing with a filtrable virus. The results would have carried more conviction if one of the denser filters like L7 had been used. The diameter of the pores is not the only factor which determines whether a given bacillus will pass through a filter. Both the filter and the bacillus carry a negative charge and only negatively charged particles will pass through a negative filter. But the pH of the medium and the presence of polyvalent ions may change the potential of the organisms and absorption of proteins from the tuberculous exudates may change the nature of the porcelain surface. Under these circumstances it is not surprising that widely differing results were obtained by different authors and that the experimenters who reported positive results have not been able to reproduce these results constantly. These differences may not be the result of a capricious behavior of the tubercle bacillus but may simply be the expression of our deficient knowledge of the conditions which influence filtration.

It has been suggested that the positive results can be explained by the occasional passage of an intact tubercle bacillus. This is by no means impossible. The bacterium pneumosintes of Ohtaky and Gates and the Schizosaccharomyces filtrans of Lewis are frankly filter passing. Avian diphtheria, Oroya fever, contagious pleuropneumonia of cattle and scarlet fever were at one time described as caused by filtrable viruses but are now recognized as the result of bacterial infection. The opposite has also occurred. Hog cholera was formerly supposed to be the result of infection with the hog cholera bacillus but is now considered as due to a filtrable virus.

There are however important reasons for not lightly dismissing the possibility of a filtrable form of the tubercle bacillus. Most of the authors who have affirmed the existence of this form report that it is usually of very low virulence and only regains its virulence after many animal passages, if at all. In experiments in which guinea pigs were infected with one single

baeillus this has not been observed. If the guinea pigs became tuberculous, the bacilli caused the usual form of disease. Vaudremer describes the symptoms caused by his filtrable virus as resembling Poncet's syndrome. Vasiliu and Irimimou<sup>14</sup> found a filter-passing form of Koch's bacillus in the glands of patients suffering from lymphogranuloma. In this relation it is interesting to remember that Falk<sup>15</sup> found a difference in electrophoretic potential between virulent and avirulent strains of pneumococci. A similar difference in the tubercle bacillus could influence their filtrability.

The possibility of spontaneous infection must always be considered in experiments with the tubercle bacillus. Calmette<sup>16</sup> states that in his experiments the acid-fast organisms were found principally in the tracheobronchial lymph nodes, but no primary effect existed at the point of inoculation. This points to the possibility of an infection by air, either by the tubercle bacillus or by an organism similar to the timothy bacillus as suggested by Cooper and Petroff.

At present it seems impossible to choose between these different possibilities and further experiments will have to decide if a filtrable form of the tubercle bacillus exists and whether Much's granules or an ultramicroscopic virus constitutes this form.

#### REFERENCES

- 1 Fontes. *Memorias do Inst Oswaldo Cruz* 2 1, 1910
- 2 Vaudremer. *Compt rend Soc de biol* 89 80, 1923
- 3 Valtis. *Compt rend Soc de biol* 90 9, 1924
- 4 Arloing and Dufour. *Compt rend Soc de biol* 94 46, 1925
- 5 Mellon and Jost. *Proc Soc Exper Biol & Med* 24 743, 1927
- 6 Cooper and Petroff. *J Infect Dis* 43 200, 1928
- 7 Montemartini. *Bull de l'Inst sierother Milan* 4 1, 1925
- 8 Fessler. *Centralbl f Bakteriologie* 98 148, 1926
- 9 Rossetti. *Bull de l'Inst sierother Milan* 6 111, 1927
- 10 Ruys. *Nederl Tijdschr v Geneesk* 72 795, 1928
- 11 Rivers. *Filtrable Viruses*, p 86
- 12 Rosenthal. *Ztschr f Hyg* 60 169, 1908
- 13 Mudd. *J Bacteriol* 8 459, 1923
- 14 Vasiliu and Irimimou. *Compt rend Soc de biol* 94 163, 1925
- 15 Falk, Gassin, and Jacobson. *J Infect Dis* 37 481, 1925
- 16 Calmette. *L'Infection bacillarie et la Tuberculose*, p 157, 1927

—G B W (C H B)

## CORRESPONDENCE

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*To the Editor*—In an article in the November, 1928, issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE entitled "The Rôle of Complement in Health and Disease" by L. J. Hadjopoulos, M.D., and Reginald Burbank, M.D., from the Laboratories of the Beth Israel Hospital, New York City, the authors, after making a reference to the work of Dangera, published in 1900 make the statement in regard to complement—"Subsequent literature offers only fragmentary or indirect evidence of its rôle as a protective mechanism." Also they make a further statement that this particular phase of the subject had been 'hitherto neglected.' I beg to point out that important work and observations along this line of investigation had been published by W. C. Gann in 1914, by Hiram D. Moore, in 1919, and by Rockwood and Beeler in 1924. Moreover, the experiments, observations and conclusions of Drs. Hadjopoulos and Burbank parallel to a very considerable extent those to be found in an article entitled 'Complement in Health and Disease' by the writer, published in the Canadian Medical Association Journal in April, 1926.

(Signed) FRED T. CADHAM

Winnipeg, Canada, Dec. 11, 1928

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*To the Editor*—I regret that in our article "The Rôle of Complement in Health and Disease" due credit was not given for an excellent article along similar lines by Doctor Fred T. Cadham of Manitoba, but at the time our work was first prepared for publication and read before a New York medical group, Doctor Cadham's article had not been published, and no further search of the literature was made after that time.

The work was merely presented as our research during a period of some nine years prior to 1926.

(Signed) REGINALD BURBANK

New York City, Dec. 26, 1928



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## GENERAL LETTER NO 1

The Program Committee earnestly requests the membership of the Society to consider and plan now the papers to be presented at the coming meeting and, in order that the program may be well balanced, of general interest, and in keeping with the prestige of the American Society of Clinical Pathologists the Committee requests

1 That all those desiring to present a paper will notify the Chairman as early as possible even if only the subject and not the title can be stated

2 That, at least two months before the time of the meeting, five copies of the title and abstract of the paper be furnished the Chairman

3 That, in so far as is possible, the papers represent original work of scientific or general interest

4 That it be specifically stated whether or not lantern slides will be used

The Committee hopes that there will be a very general response to these suggestions

The duration of the meeting and the size of the membership make it obvious that, perhaps, not all who desire to do so will be able to present their papers. The earlier the response, however, the better will be the opportunity for a place on the program

ROBERT A KILDUFFE, M D  
CHAIRMAN, PROGRAM COMM  
ATLANTIC CITY HOSPITAL  
ATLANTIC CITY, N J

## GENERAL LETTER NO 2

Preparations are being made for a Scientific Exhibit at the Portland meeting of the American Society of Clinical Pathologists. Undoubtedly many of our members have some interesting specimens, apparatus, or technical methods that they may desire to show to the Fellows at the Convention.

We shall appreciate any cooperation on your part in making this feature of our annual meeting a source of instruction to our membership. Kindly let me know what we may expect from you in this field so that we can make the proper arrangements for its display.

E D DOWNING M.D.

CHAIRMAN COMMITTEE ON EXHIBITS

WOODMAN SANITARIUM

WOODMAN COLORADO

## GENERAL LETTER NO 3

At the last meeting of the Society in Minneapolis it was unanimously agreed that there would be an annual award of a prize to be bestowed on the member of the Society who shall have made the most valuable and original contribution in the field of clinical pathology, this prize to be called "The Ward Burdick Award of the American Society of Clinical Pathologists." The nature of the award shall be decided on and the winner selected by the Research Committee. For this year, at least, the award is to be given irrespective of the subject studied, although possibly in later years other committees may see fit to limit the field to research on certain subjects.

It is hoped that many of our members will submit their work for the consideration of the Research Committee at the same time that the papers are sent to the Program Committee so that ample time is allowed for mailing the same to the various members of the Research Committee for their perusal. No papers will be accepted from applicants later than two months before the next annual meeting in Portland. Your Committee has deemed it advisable at this time to allow any member, whether conducting a small laboratory or working in a large institution to compete for the prize realizing as we do that the individual efforts and originality are the basis for estimation of merit.

The Committee has been considering the advisability of endeavoring to foster the study of certain conditions such as "Undulant Fever," "Agranulocytosis," "Acute Leucemia," by our members throughout the country, with the idea of suggesting to the President and the Program Committee that a half day be given over at our next meeting for papers on one of the above subjects followed by a round table discussion on the matter. It is suggested that by such a plan many of our members who are so situated that they cannot devote much or any time to a definite problem of research, may be able to bring in good case reports and experiences which, when collected, will be of great mutual benefit. If such plans are ultimately agreed upon it is hoped that ample photographs, slides or pathologic material will be presented for a thorough discussion of any case or cases described. Any suggestions in regard to the above method or procedure or other matter of research will be gratefully received by your Committee.

ALVIN G. FOORD M.D.

CHAIRMAN OF RESEARCH COMMITTEE.

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THE REGISTRY OF TECHNICIANS—PROPOSED WORKING SCHEME

I. NAME

1 The Registry shall be known as The Registry of Technicians of the American Society of Clinical Pathologists and shall be directed by a Board of Registry of six members appointed by the Society.

II. OBJECTS

1 The objects of the Registry shall be

- a To establish the minimum standards of educational and technical qualifications for various technical workers in the clinical, research and public health laboratories

- b To classify them according to these standards
- c To receive applications for registration and issue a certificate of registration to those who meet the minimum standards of requirements
- d To register schools which offer an acceptable course of laboratory training
- e To conduct a placement bureau for registered laboratory technicians
- f To cultivate a high ethical standard among laboratory technicians in accordance with the code of ethics established by the American Society of Clinical Pathologists

### III BOARD OF REGISTRY

1 The Board of Registry shall be composed of six members elected by the American Society of Clinical Pathologists, two members to be appointed by ballot to serve for three years at each annual meeting of the Society or until their successors have been elected. The first Board shall consist of six members, two of whom shall be elected for a term of one year, two for a term of two years and two for a term of three years. It shall elect its own chairman from among the holdover members and Secretary Treasurer.

2 The Board of Registry shall be authorized to employ a director who is empowered to manage the affairs of the Board.

3 The duties of the Director shall be to administer the office of the Board by taking charge of registration of technicians, issuance of certificates, and conducting a placement bureau and such other business as may be necessary to carry out the functions of this Board. He shall be directly responsible to the Board.

### IV CLASSIFICATION OF LABORATORY TECHNICIANS

1 Technical workers in the clinical research or public health laboratories shall be classified according to their education, training and experience, as follows:

- a Medical Technologist
- b Laboratory Technician

2 Medical Technologist shall signify one who possesses a university degree with at least one year in basic sciences including chemistry, bacteriology, physiology and pathology with laboratory demonstration or credit equivalent to the same as determined by the Board and at least one year of practical experience in a recognized laboratory, devotes himself wholly to the technical work of a medical laboratory, and has rendered a valuable service in the field of laboratory medicine through research, teaching or other scientific endeavors. Medical Technologists shall be elected annually by the unanimous vote of the Board of Registry. A laboratory technician who possesses no college degree but who has rendered long and faithful service in a recognized clinical, research or public health laboratory in a responsible capacity, may be eligible to this designation.

3 Laboratory Technician shall signify one who is fully qualified to render general or special technical service in a clinical, research or public health laboratory under the supervision of a qualified director, and shall exhibit the following minimum preparation and qualification:

- a Graduation from an accredited high school
- b One year of didactic work in basic sciences including chemistry, bacteriology, physiology and pathology, together with laboratory demonstration, or credit equivalent to the same as determined by the Board
- c Six months of actual experience in a recognized clinical, research or public health laboratory

4 Laboratory Technician or Medical Technologist who limits his work in a certain special field shall be so designated as Bacteriological Laboratory Technician, Chemical Laboratory Technician, Public Health Laboratory Technician, etc., in the case of laboratory technicians and bacteriologist, serologist, parasitologist, etc., in the case of medical technicians.

## V REGISTRATION OF TECHNICIANS' CERTIFICATES

1 Candidate shall properly fill out an application blank of the Registry and file with the Director of the Board of Registry

2 A registration fee of three dollars shall accompany the application. This will be returned if the application is rejected

3 Annual renewal of the certificate is required for which a fee of one dollar is charged

4 Upon the receipt of application the Director shall conduct a preliminary investigation of each applicant and the result shall be filed with the application. Certification of applicants shall be done by the Board of Registry at the annual meeting

5 A certificate of registration shall be issued to all applicants accepted by the Registry

6 A certificate may be revoked at any time for cause by order of the Board. A hearing may be granted on request

## VI EXAMINATION

1 A formal examination may be deemed necessary by the Board to determine the qualifications of an applicant in which case written oral and practical examinations shall be conducted at a place and by a member of this Society as arranged by the Director of the Board

2 An additional fee of \$10.00 to cover the expense shall be charged the applicant

## VII REGISTRATION OF SCHOOLS FOR LABORATORY TECHNICIANS

1 The Board shall investigate classify and periodically inspect through an accredited representative the schools and laboratories which conduct a training course for laboratory technicians

2 These schools and laboratories may register with this Board and receive an annual certificate of registration provided the course of training given meets the approval of this Board. An annual registration fee of one dollar shall accompany the application

## VIII PLACEMENT BUREAU

1 Registered technicians and technologists may upon proper application be placed through this Bureau operated by the Board

2 A fee equivalent to five per cent of the first month's salary shall be charged to the technician who obtains employment through this Bureau

## IX. CODE OF ETHICS

1 All registered technicians and technologists shall be required to strictly observe the Code of Ethics as defined by the American Society of Clinical Pathologists, namely that they shall agree to work at all times under the supervision of a qualified physician and shall under no circumstances on their own initiative render written or oral diagnoses except in so far as it is self evident in the report or advise physicians and others in the treatment of disease or operate a laboratory independently without the supervision of a qualified physician or clinical pathologist

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News and Notes

The Registry of Technicians of the American Society of Clinical Pathologists is now functioning quite satisfactorily considering the short period that it has been established. The aims and objects of the Registry are presented on another page

The establishment of the Registry will prove a great boon both to technicians as well as to the hospitals and clinical pathologists. In this age of efficiency and mass production the rise and growth of this handmaiden to the physician will be of incalculable value in the saving of human lives by placing properly qualified technicians in localities where there are none at present

Technicians are rapidly availing themselves of the opportunity to obtain certificates in the Registry, thus raising the status of their craft to an elevated and respected position

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Members will kindly report any change in address to the Secretary of the American Society of Clinical Pathologists, Metropolitan Building, Denver, Colorado

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The Service Bureau of the American Society of Clinical Pathologists has also been active in making contacts between pathologists seeking a change in location and institutions desirous of engaging their services

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The next annual meeting of the American Society of Clinical Pathologists will be held in Portland, Oregon, July 5, 6, and 8, 1929. All members are urged to make plans to attend this convention which besides promising a great intellectual feast will also enable them to visit our great far west

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The attention of the members of the American Society of Clinical Pathologists is called to the fact that at our next convention in Portland, Oregon, one half day will be devoted to a group presentation and general discussion of the subject of "Undulant Fever." *Participation in this symposium by all the members is earnestly requested by the Program Committee and Research Committee*

# *The Journal of Laboratory and Clinical Medicine*

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## AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

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### THE WARD BURDICK RESEARCH AWARD OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

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THE yeoman services of Dr Ward Burdick in helping to organize the American Society of Clinical Pathologists and as its Secretary to raise it to an enviable status, have led the members and the administrative officers to establish a memorial which will perpetuate his endeavors for the organization

Dr Ward Burdick assisted at the birth of the American Society of Clinical Pathologists in St Louis on May 22 1922 at a call that he sent out for a meeting which was destined to be the first successful national convention of clinical pathologists On that occasion he was nominated temporary secretary and then became the permanent incumbent of this office when the Society was definitely formed Dr Burdick was eminently suited for this position Of a calm and judicial temperament painstaking and meticulous in the conduct of the affairs of the Society, imbued with a spirit of idealism which led him to devote all his spare hours in reflections how best to promote the progress of the organization Dr Burdick was a providential selection for the position in the early days of the organization when the crusading spirit was needed to gather forces and battle the evil spirit of commercialism that was beginning to infiltrate the clinical pathologists of the country

He was never too busy to answer a call in behalf of the Society Although his attendance at the meetings was fraught with peril to his health he was present at the earlier conventions and took part in the deliberations even though he was suffering from a fever at the time

It is only natural therefore that the Research Committee of the American Society of Clinical Pathologists for the year 1927-28 selected the idea of a medal as a fitting remembrance of one who had served the Society so conscientiously and as an example for the younger men to emulate the spirit of idealism, of progress and of research which was a dominant feature of

Ward Burdick's character. The Committee, consisting of Dr H J Cooper Chairman, Dr H J Nichols, Dr H S Martland, Dr Kenneth Lynch, Dr Wm C MacCarty and Dr W G Lyle, recommended to the Society that a fitting award be established to commemorate the name of Dr Burdick, the prize to be in the form of a medal to be presented annually for some outstanding research project in the field of clinical pathology by a member of the Society. The present Research Committee appointed by Dr Frank W Hartman, President of the Society, consists of Dr Alvin G Food, Chairman, Dr Oscar B Hunter, Dr Stanley P Reimann, Dr W G Gamble, Jr, and Dr A S



Giordano. It was the unanimous opinion of this committee that the award should consist of an appropriate gold medal bearing the profile likeness of Dr Ward Burdick and the nature of the award on its face and on the reverse side the seal of the Society and the name of the recipient as well as the date of presentation.

The award is to be presented to the successful candidate by the President of the Society at the annual meeting on the evening of the banquet. The competition is now open to all the members of the American Society of Clinical Pathologists. All candidates for the award must present their theses at least two months prior to the annual meeting in order that they may receive the careful judgment of the Research Committee.

## ETIOLOGY OF ACUTE LEUCEMIAS\*

By A S RUBNITZ MD OMAHA, NEBR

IN THE last six years I have seen five cases of acute leucemia. Being greatly impressed with the similarity of the course and clinical picture of these cases and the still more striking meagerness of information regarding the etiology of the disease, it seemed worth while to attempt to systematize all the data on the etiology of leucemia.

I will not burden the audience with the detailed histories of these cases as they were all typical cases of acute leucemia. Four of the patients were males of the following ages six fifteen twenty and sixty six one was a female aged fifty nine. Their leucocyte counts ran from 60,000 to 300,000, for a brief period only did the leucocyte count in one case (now aged six) drop to 4,000 that happened shortly after extensive radiation. But soon after, the leucocyte count increased reaching 100,000 before death. Clinically they all had in a greater or lesser degree the usual triad of symptoms sepsis, necrotic inflammation of the oral cavity, and hemorrhages.

On first observation each one of them was apparently suffering from an acute infection. The questions to be answered were Did the infection produce the leucemia, or was the leucemia produced by some other unknown factor the infection being secondary to the lessened resistance of the tissues? I shall endeavor to answer these questions.

The literature on the subject of leucemia has been in the process of being written for the last eighty years. A more or less definite conception about this disease has been worked out and accepted by our textbooks but even the well accepted orthodox views are not final and I dare say the near future will see the chapter on leucemias rewritten.

Our present day classification divides the leucemias (both acute and chronic) into the lymphatic or lymphogenic and the myeloid or myelogenous.

The basis for this classification is the dualistic theory of Ehrlich. According to this theory all the leucocytes are divided into two main groups the granulocytic and the nongranulocytic. The cells of the first group have their origin in the bone marrow while the cells of the second group originate in the lymphoid tissue. A leucemia therefore is classed as myelogenous if the majority of the cells determining the diagnosis of leucemia are of the young granulocytic type. The same holds true for the lymphogenic varieties.

This strict differentiation of leucemias may be fitting in the chronic types since the blood as well as the clinical findings and to a certain extent, the clinical course differ perceptibly in the two chronic varieties.

\*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists Minneapolis Minnesota June 5 9 and 11 1919



In acute leucemias, on the other hand, we do not have any appreciable differences either in the clinical or in the laboratory findings. Clinically all the acute leucemias are alike. They are all characterized by the same triad of symptoms, run the same clinical course, and have the same fatal termination.

The differentiation into acute lymphatic and acute myeloid leucemia is based on the supposedly different blood pictures. But does there exist such a sharp differentiation?

Some hematologists make matters even more complicated. They recognize a triple origin of leucocytes: (1) Bone marrow (polymorphonuclear cells and their progenitors, the myelocytes), (2) reticulo-endothelial system (monocytes), and (3) lymphoid tissue (lymphocytes and their progenitors, the lymphoblasts). Following this classification of the origin of leucocytes, they recognize a third variety of leucemia, namely, the monocytic leucemia (Reschad and Schilling<sup>1</sup>).

It may not be out of place to mention here the views advanced by Pappenheim,<sup>2</sup> the chief exponent of the unitarian theory of the origin of leucocytes. According to him, all the leucocytes have their common origin in one cell, the large lymphocyte, and only through special differentiation do we get the two main classes of cells: granulocytic and agranulocytic.

When one studies mature cells he will be more inclined to accept a theory which supposes that the different leucocytes have different origins, but if one is given to the study of immature, embryonal cells, the unitarian theory of Pappenheim is more likely to appeal to him.

In acute leucemias we are always dealing with immature, embryonal cells. The presence of immature cells in the blood is perhaps the most essential requisite for the diagnosis of the disease.

The diagnosis of acute leucemia usually hangs upon a large mononuclear cell, which used to be considered in the old textbooks as a large lymphocyte. The cell usually has a vacuolated nucleus, with several nucleoli. There is marked unevenness in the density of the nucleus, granules are usually absent. In some cells the entire nucleus has a network of stringy matter. It is a cell one will always remember, once one has seen it. It is not a large lymphocyte, nor is it always a myeloblast. It is a very young form of a leucocyte, which is sometimes hard to place in the scale of classification. If you will consider it a very young lymphocyte or lymphoblast you may come just as near to the truth as considering it a young myelocyte or myeloblast.

Some hematologists consider the oxydase reaction the most deciding factor in differentiating the granulocytic cells (beginning with the myeloblast, down to the mature polymorph) from the agranulocytic cells (beginning with the lymphoblast, down to the mature small lymphocyte).

All the granulocytic cells, having their common origin in the bone marrow (according to accepted theories) give a positive oxydase reaction because they contain some ferment in them.

But the ferment content fluctuates with the maturity or immaturity of the cells. A very young myeloid cell, a myeloblast, or even a promyelocyte, may not give this reaction, so that only a positive oxydase reaction is of value, while a negative one does not mean much. In other words, we are often deal-

ing with a cell in acute leucemias which may be considered either a lymphoblast or myeloblast, depending on the morphologic interpretation of the observer. The type of leucemia, then, would be classed according to the interpretation.

Barchasch,<sup>3</sup> in summarizing his paper on leucemias, makes the following statement: "The protease or oxydase reaction is necessary in the differential diagnosis of lymphatic and myeloid leucemias." Yet in Case 3 of his report, he did not get a positive oxydase reaction, although he considers it as a case of myeloid leucemia. In discussing that case he remarks: "It is interesting that the myeloblasts of the given case, irrespective of the fact that they were so typical (myeloblasts), did not give the oxydase reaction, this can be explained on the basis of their belonging to the youngest forms (embryonal types)."

After all, a theory is in the majority of instances a logical explanation of observed or recorded facts. With all due respect to the authorities behind the dualistic or triadistic theories, the usual blood findings in acute leucemias warrant the acceptance of the unitarian theory of the origin of leucocytes.

Some other published data seem to substantiate the latter theory.

1. Pappenheim<sup>4</sup> calls attention to the following fact:

"In some lower invertebrates which possess neither bone marrow nor lymph glands we may still find both forms of cells in the blood: the granulocytes as well as the agranulocytes."

2. Ellermann and Bang's deductions (cited by Schmeisser<sup>4</sup>) on experimental leucemias in the fowl:

"Spontaneous and transmitted leucemias occur in two types: Myeloid and lymphatic. A myeloid leucemia may occur in the first generation, and a lymphatic in the next; or both types may occur in the same generation."

3. Parker and Rhoads<sup>5</sup> in reporting their own as well as other observers' findings on incubated leucemic blood record the following interesting observation:

"New embryonal cells (designated by the authors as 'X' and 'Y' cells) were developed from the blood of either cases. Myeloid or lymphatic leucemias. These cells vary from strongly oxydase positive to oxydase negative. Without going into further discussion on the origin of leucocytes, the logical deduction from the presented data would be: To Drop the Heretofore Accepted Subdivision of Acute Leucemias. We Should Consider a Case of Acute Leucemia as an Entity in Itself Irrespective of the Possible Origin of the Cells: Myelo- or Lymphogenic."

Of my five cases the first could have been diagnosed as septicemia due to mouth infection; the second had "canler sores" in the mouth. The third did not have any apparent oral infection, at least at the time I examined him, but the fourth was a very outspoken case of gangrenous stomatitis. The fifth started with a "sore throat" and when seen by me had a continuous hemorrhagic condition of the oral mucous membrane.

Mouth infection therefore was apparent in 80 per cent of the cases. In all cases of acute leucemia reported in the literature one will find mouth infec-

tion present in at least 60 per cent. One naturally will be led to think about the possibility of infection as the etiologic factor in this disease.

Ellerman and Bang (cited by Schmeisser) reported that they succeeded in transmitting leucemia from a spontaneous case in a fowl into other healthy birds. They used a filtrate of the spleen, liver, and lymph glands. They concluded that a cell-free filtrate is effective, and that, therefore, the cause must be an organized virus. In 1909 the same authors reported the transmission of the disease into the sixth generation with a Berkefeld filtrate. Their conclusions were (a) Leucemia must be an infectious disease (b) It is a disease due to a filtrable virus. Other investigators at that time were also successful in reproducing the disease with Ellerman and Bang's filtrate. Schriddle<sup>1</sup> questioned the deductions of Ellerman and Bang. He claimed that he produced changes similar to those of Ellerman and Bang by the injection of an extract of an entirely normal organ. In this country Schmeisser<sup>2</sup> reported his successful results in transmitting leucemia to the fowl using the methods of Ellermann and Bang. While all the above experiments point to infection as the etiologic factor in leucemia, yet no definite organism has been claimed by any observer to be the causative one. A variety of organisms have been found by different authors in association with this disease: typhoid-colon group, diphtheria bacillus and diphtheroids, Vincent's organisms, etc. It is generally accepted, though, that no known microorganism is responsible for the disease. Vincent's organisms are perhaps most commonly found in the oral cavities of acute leucemic patients, especially if there is any necrosis of the mucous membranes, but Vincent's organisms may be found in any condition of the mouth where sloughing is taking place. Therefore, while most of the leucemic patients suffer from a necrotic condition of the oral mucous membrane, yet we have very little evidence to suspect that leucemia is caused by any specific organism.

Let us accept for a time a different view. That leucemia is primarily a malignancy of the blood-forming tissues. We do get a hyperplasia of the splenic pulp, lymphoid tissue, bone marrow, and the reticulo-endothelial system throughout the body. Assuming that the hyperplasia and to some degree the metaplasia occurring in leucemia are the primary conditions caused by the same possible agents that are responsible for malignant growths in other tissues, assuming further that the malignant cells have multiplied in great enough numbers to poison the blood stream and develop a state of cachexia in the patient, we would be dealing then with a marked lowering of the resistance of the tissues to infection and almost any organism would act as a secondary invader.

The latter theory would not be very logical because we always find that the patient's health has been good until some infection developed which culminated in leucemia. The third possible theory would be that we are dealing with neither a malignancy of the blood-forming organs nor a specific infection, but rather with a special condition brought about or precipitated either by an infection or by an abnormal metabolic state.

Waid,<sup>8</sup> for instance, in his revision of the literature found fifteen cases of leucemia during pregnancy and four in which the disease was recognized soon after parturition.

Accepting the last theory we would define leucemia as *an unusual blood reaction to certain toxins* the toxins may be produced by various agents such as bacterial infections or metabolic derangements. The fact that we mostly find sloughing mucous membranes of the mouth associated with the disease would suggest the possibility that *necrotic mucous membranes may contain a toxic substance which is the causative agent*.

Schultz and Veise<sup>10</sup> described a new disease which they named 'agranulocytosis'. This unusually fatal disease in many respects resembles acute leucemia. The total leucocyte count is generally very low, it may be below one thousand per cubic millimeter. The lymphocytes are relatively very high, while the granulocytes are pushed out of the picture until, before death they disappear altogether from the peripheral circulation.

This disease should not be confused with leucemia because we do not find there the immature embryonal leucocytes. We are dealing rather with a condition in which the granulocytes are destroyed in the blood stream, when all of the granulocytes or most of them are gone, we have a lethal termination. The point of similarity between the two diseases is the necrosis of mucous membranes. The latter always occurs in agranulocytosis and necrosis of the oral mucosa, vaginal mucosa or even the intestinal mucosa has been described in connection with this disease.

It would seem logical to assume that the toxin in both agranulocytosis and acute leucemia is of the same quality differing only in quantity.

In agranulocytosis the condition is much more active, the toxin is liberated in quantities sufficient to destroy the granulocytes in the blood stream. The hemopoietic system does not have any time to attempt any comeback and the individual succumbs without a battle. We do not find therefore in such cases any perceptible histologic changes in the lymphoid tissues, bone marrow, spleen, etc.

In acute leucemia the quantity of the toxin is less. The granulocytes are destroyed in the blood stream but not as fast as in the first condition. The blood forming organs are stimulated to activity, with the result that numerous immature cells of every description are thrown out in the circulation. The condition lasts longer although the outcome is usually the same. I say 'usually the same' because we could picture to ourselves cases in which the toxin is liberated in insufficient quantities to kill the individual and a balance of health might be reestablished.

It is generally accepted that any case that does not go on to a fatal termination should be excluded from the category of acute leucemias. This postulate may not always be true. Herz<sup>11</sup> reported a case and cited several others in which the blood picture was very suggestive of leucemia, clinically these cases suffered from an angina and necrotic condition of the mucous membranes of the palate and tonsils. The cases reported by Herz all recovered. His conclusion therefore is that leucemias are not all fatal.

The condition known as "infectious mononucleosis," which is most generally found in association with Vincent's angina, should also be included at the bottom of the list

Speaking of septic infections secondary to necrotic inflammation of mucous membranes, Weiss<sup>12</sup> expresses the following views

1 A septic infection may produce in some instances a simple monocytic reaction (monocytic angina, infectious mononucleosis)

2 A septic infection may exert its action on the blood-building tissues resulting in a hypogranulocytosis, without any multiplication of the general leucocyte count (symptom complex of Schultz), and lastly

3 A septic infection may produce a definite increase in the mononuclear leucocytes, with a hypogranulocytosis at the same time (acute leucemia)

All the three kinds of reactions have one thing in common, namely, necrotic inflammation of mucous membranes

His deductions are that the hypogranulocytosis or agranulocytosis is not the cause of the necrotic inflammation of the mucous membranes, but both are disease symptoms, and the necrosis of the mucous membranes and the blood findings are caused by the septic infection

But Weiss does not attempt to explain why the septic infection produces such a peculiar reaction in this type of cases. His deductions are not very clear. The point to be emphasized is that in septic infection of mucous membranes we get this peculiar reaction of the body which brings about the above-described clinical entities. Since we cannot ascribe these conditions to any specific infection, we conclude, therefore, that the cause must lie in the toxic substances liberated by necrotic mucous membranes. The toxin is very likely a definite chemical entity, a leucocytotoxin, it is lytic to granulocytic cells. The developing clinical superstructure depends on the quantity and rapidity of liberation of this particular toxin.

Agranulocytosis, acute leucemia, or infectious mononucleosis may all be produced by the same leucocytotoxin.

The different modifications and fluctuations of the number of leucocytes in leucemias depends very likely on the amount of toxin circulating in the blood stream.

Szillard<sup>13</sup> (cited by Barchasch) attempted to explain the fluctuation of the number of leucocytes in leucemic and pseudoleucemic bloods. He incubated a known quantity of the patient's leucocytes in the patient's serum, he then counted them after twenty-four hours' incubation. He found that in cases of subleucemic condition (where the number of leucocytes was low) the resistance of the leucocytes was low, while in cases where the number of leucocytes was high, the resistance of the leucocytes was much greater. His conclusions were that leucemic, subleucemic, or aleucemic conditions depended on the resistance of the leucocytes.

He failed, however, to take into consideration another possible factor, namely, that the cells were incubated in the patient's serum. The serum could carry in it a leucocytolytic substance which was responsible for the variation in the resistance of the leucocytes.

## SUMMARY

1 There is no logical reason for the division of acute leucemias into lympho and myelogenic, because

- a Clinically they represent one and the same entity
- b Microscopically we cannot always draw a line between lymphogenic and myelogenic cells. The oxydase reaction is only of value if positive

2 Acute leucemias while always associated with infection, are not caused by any specific microorganisms

3 Necrotic inflammation of mucous membranes is usually associated with acute leucemias as well as with the condition known as "agranulocytosis"

4 Some obscure toxin liberated by necrotic membranes, by nature a leucocytolysin, is very likely responsible for both acute leucemia and agranulocytosis

5 Acute leucemia and agranulocytosis may be caused by a toxin of the same quality. The difference in the body reaction is quite likely due to quantitative variations in toxins produced and liberated

## REFERENCES

- 1 Reschad H and Schilling V. Ueber eine neue Leukämie durch echte Uebergangsformen (Splenocytenleukämie) und ihre Bedeutung für die Selbstständigkeit dieser Zellen. *München med Wchnschr* 60 1981 84 1913 (Quoted by Barchanisch)
- 2 Pappenheim A. Atlas der menschlichen Blutzellen. Jena G Fischer, pp 81 1906
- 3 Barchanisch P. Clinic of Acute and Chronic Leukemia, 6 cases, *Russk klin* 7 696 71 1927
- 4 Schmeisser, H C. Leukemia of the Fowl. Spontaneous and Experimental. *Johns Hopkins Hosp Rep* 17 551 584 1916
- 5 Parker E Jr, and Rhoads C P. Some Observations on Incubated Leukemia Bloods. *Am J Path* 4 167 180 1928
- 6 Schriddle, H. Gibt es eine Aetiologie der Leukämie? *Deutsche med Wchnschr* 35 280 1909
- 7 Ward, G. The Infective Theory of Acute Leukemia. *Brit J Child Dis* 14 10 20 1917
- 8 Ordway, T. and Gorham L W. Leukemia, Oxford Medicine. London and Toronto Oxford University Press 2 681 762 1920
- 9 Schultz, W. Gangränisierende Prozesse und Defekt des Granulozytensystems, *Deutsche med Wchnschr* 48 1495 1496 1922 (Quoted by Barchanisch)
- 10 Verso. Pathologisch anatomische Befunde bei der Agranulozytose. *Deutsche Med Wchnschr* 48 1406 1437 1922 (Quoted by Barchanisch)
- 11 Herz A. Infektionen mit leukämischen Blutbild, *Wien klin Wchnschr* 39 837 841 1926
- 12 Weiss J. Ueber die gegenseitigen Beziehungen zwischen Schulz'schem Symptomenkomplex (Mucositis necroticans agranulocytica) akuter Leukämie und septischem Infekt. *Wien Arch f inn Med* 14 303 334 1927
- 13 Szillard P. Ueber die aleukämische Myelose. *Deutsche Arch f klin Med* 144 286 296, 1924

# RESULTS IN VARIOUS DISEASES FROM ELIMINATION OF FOCI OF INFECTION AND USE OF VACCINES PREPARED FROM STREPTOCOCCI HAVING ELECTIVE LOCALIZING POWER\*

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THE results from a long series of experiments on focal infection and elective localization reported during the last fourteen years have shown that (1) foci of infection, wherever found, commonly harbor the causative organism of various systemic diseases, (2) they afford favorable conditions for the entrance of bacteria and their toxic products where bacteria tend to acquire and maintain relatively high or peculiar invasive powers and (3) the situation, aside from predisposing factors and hence to a large degree the character of the systemic disease depends on the specific or elective localizing power of the infecting organisms in most instances streptococci. This statement, based on first-hand knowledge of clinical conditions, the bacteriology involved and animal experiments, is considered a good working hypothesis in clarifying the confusion which still exists in the minds of many regarding the application of the underlying principles involved. Even Holman, after his critical review of the work on focal infection and elective localization, in which he conceded the great importance of the former, yet regards proof of the latter as insufficient, stated "However, what Rosenow and his followers particularly showed and what all other investigators of the problem have definitely demonstrated is that streptococci do localize in various organs and tissues and can produce lesions at least sufficiently suggestive of those found in man so that their potential danger in infected foci cannot be neglected."

The large number of instances reported in the literature during this period (fourteen years) in which causal relationship between focus and systemic disease is indicated by the clinical history and improvement or disappearance of the systemic condition following removal of foci of infection such as occur commonly in tonsils, teeth, sinuses, intestinal tract, prostate and cervix would alone be convincing if it were not that a considerable number of patients fail to improve following removal of one focus or more. Such failure, however, should not be interpreted as nullifying the principle involved, for there are good reasons why clinical improvement may not follow the removal of one or more foci of infection. The disease may have existed for so long a time as to produce irreparable harm or it may have become so thoroughly established as to continue independent of a primary focus. The particular focus or foci removed may not have had etiologic significance at the time of removal, or even before, or as in the case of dental or tonsillar foci, the opera-

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tive procedure may have been inadequate. The responsible focus may have been overlooked because so often symptomless. Not all systemic diseases are expressions of focal infection. Clinical improvement occurred with such regularity and was so marked, however, following removal of foci of infection in those cases in which the results in animals in our experiments had demonstrated the presence of the causative organism as to leave no doubt of the great value of this procedure in suitable cases. We believed much valuable information might be obtained if clinical manifestations were studied in conjunction with first hand knowledge of the bacteriology and the results obtained in animals following injection of cultures freshly isolated from suspected areas of infection or tissues involved. Moreover, since the injection into animals served so often to separate saprophytic from pathogenic or causative strains commonly indistinguishable by cultural means, and since in a few cases marked improvement was noted following the use of vaccines prepared from the bacteria isolated from the systemic lesions of injected animals we determined also to study the therapeutic effect of these truly antogenous vaccines. The excellent clinical and laboratory facilities afforded at The Mayo Clinic and The Mayo Foundation are particularly well adapted for such study.

Most of the patients included in this report were seen by one or both of us with various members of the clinical staff during the last six years. Many of the patients presented puzzling diagnostic problems particularly regarding the likelihood of etiologic relationship to one or more foci, then situation and *whether or not to remove suspected foci of infection*. The decision regarding any one or all of these points was not always made on clinical considerations alone but on these and the results of the animal experiments. Thus some times questionable foci were or were not removed, depending on whether or not the animals injected with material or cultures from such foci developed lesions in structures corresponding to those affected in the patient. On the basis of previous studies clinicians felt that the results in animals might throw light on the differential diagnosis in certain cases. The great majority of patients had previously sought in vain for relief from a chronic often progressive condition and therefore if what was advised to be done resulted in amelioration or relief of symptoms we could be fairly certain that this was not accidental but was due to the treatment instituted.

#### METHODS OF STUDY

The cultural methods employed were similar to those reported by either of us at various times. The condition of the patient at the time of study was also considered. A higher incidence of localization and more marked lesions in animals were obtained if cultures were secured at the time of acute systemic manifestations or during exacerbations of symptoms in chronic conditions than those obtained during quiescent intervals. Great care was exercised in the collection of material for study both as regards the clinical observations and the prevention of contamination by extraneous organisms.

If animals were injected intracerebrally in case of disease of the central nervous system and if they were injected intravenously in case of other diseases and symptoms or lesions developed similar to those in the patient from



whom the culture was obtained, the causative organism, nearly always a green-producing streptococcus, was regularly recovered in pure culture or with only scattered colonies of other bacteria, from the injected animal. The vaccine used for treatment was usually prepared from the streptococcus thus isolated and in most instances represented the first, more rarely the second, and seldom a higher subculture. In some cases the vaccine administered was prepared from heterologous strains of the streptococcus which had produced symptoms in animals simulating those of the patient. In other cases, such as furunculosis it was prepared directly from the freshly isolated bacteria without injection of animals.

Various methods have been used in preparing the vaccine. Many consisted of the killed twenty-four hour culture in glucose-brain broth or glucose

TABLE I  
PERCENTAGE INCIDENCE OF ASSOCIATED INFECTIONS\*

DISEASE	AGES	MALE	FEMALE	TONSILLITIS	INFLUENZA		FOCI OF INFECTION IN							PULPLESS TEETH	
					REMOTE	IMMEDIATE	TONSILS OF TONSIL LAR TACS	TEETH (PYORRHOIA)	SINUSITS	PROSTATE OR SPERMIAL VESICLES	CERVIX	CASES	POSITIVE (PER CENT)		
Encephalitis	205	145	57	46	37	32	70	56	2	16	2	102	87		
Arthritis	109	67	43	63	40	3	51	69	6	94	53	74	61		
Spasmodic torticollis	32	23	9	53	41	6	60	75	3	57	11	17	76		
Multiple sclerosis	27	19	8	33	41	15	78	67		37		19	89		
Chronic polyomyelitis	17	12	5	47	30	18	65	71		25		13	92		
Neuritis and myositis	17	10	7	71	35	24	41	71	24	80	57	12	42		
Gastroduodenal ulcer	27	25	2	33	37		74	74		72	100	21	52		
Lesions of eye	19	9	10	58	42	5	53	53		100	60	10	50		
Lesions of skin	23	18	5	35	26	9	74	56	9	72		13	77		
Prostatitis	24	24		42	54		6	54	4	100		13	69		

\*In computing the percentages in Tables I and II any fraction less than one half is dropped. If the fraction is one half or more the next higher figure is given.

broth, and a few of the killed cultures in pancreatic digest broth. About two-thirds consisted of the sodium chloride suspension of the density of the broth cultures made directly from the unwashed sedimented bacteria or at various intervals from those preserved in dense suspension in glycerol, two parts, and saturated sodium chloride solution, one part. One cubic centimeter of these dense suspensions was made to represent the sediment after centrifugalization of the growth from approximately 120 cc. of the eighteen to twenty-four-hour broth culture. In these, viability of some of the bacteria was preserved for many weeks, and specific immunologic properties, with few exceptions, were preserved almost indefinitely when the bacteria were kept in the ice box. The bacteria were killed by adding 0.5 per cent phenol or 0.3 per cent tricresol to the broth culture or the diluted sodium chloride solution suspension of the density of the broth culture, and if not sterile in twenty-four hours, by heating once, or at the most twice, at 60° C. for thirty minutes.

The vaccines were given subcutaneously in increasing doses once or twice a week beginning with 0.1 cc or about 200,000,000 dead bacteria and increasing gradually by 0.1 cc until 1 cc or about 2,000,000,000 were given provided the reaction in the patient remained minimal and the results obtained were seemingly favorable. The dosage was almost never increased above 1 cc. We soon learned to avoid marked local or constitutional reaction for the patient's symptoms were nearly always worse in consequence. Hence the patient's condition and reaction rather than the size of dose were used as the main guide to dosage. The succeeding dose was reduced and the interval made correspondingly longer if the one previously caused a marked local or constitutional reaction due to high toxicity of the vaccine, or to what appeared clearly to be an inherent or acquired hypersensitiveness in the patient.

#### RESULTS

The percentage incidence of associated infections, particularly focal infection, in the wide range of diseases studied (Table I) was very high. The causative streptococcus often was isolated not only from badly infected tonsils but from tonsils that appeared small and normal but which on removal were large and sometimes contained abscesses, from unsuspected remnants of tonsils, and from symptomless roentgenographically positive and negative pulpless teeth, root tips and residual areas which had not been considered previously or which were thought to be harmless. A study of the figures expressing the incidence of remote and immediately previous attacks of influenza shows that the incidence of the former while relatively high in all, does not vary greatly in the different groups whereas the latter appears to have had etiologic significance in diseases of the nervous system.

The high incidence of cervicitis in patients with neuritis and myositis (57 per cent), lesions of the eye (60 per cent) and arthritis (53 per cent) is believed of significance because wholly absent or present in only a few cases of the other groups studied.

The incidence of prostatitis associated with or without seminal vesiculitis varied from 16 per cent to 100 per cent in the different groups. The incidence of 16 per cent in the encephalitis group is certainly too low because in a large number of the earlier cases special examination was made only in those cases in which there were suggestive symptoms of prostatitis.

The interval that elapsed from the time of our study and the reply to the questionnaire varied from one to five years. Not all of the patients seen were advised to have foci removed or to take the vaccine despite the fact that in the last few years a fair number came to The Mayo Clinic because some one of their acquaintances had been benefited by this treatment. Some could not stay to have the foci removed and for one reason or another failed to have it done after returning home. Others that had one focus or all foci removed, failed to reply to the questionnaire, some were lost trace of, a few died and in others the information obtained was too meager to have statistical value. Satisfactory data were received concerning 358 (72 per cent) of the 500 patients from the attending physician from reexamination from personal letter or from the questionnaire sent to the patient.

In Table II we have summarized the results obtained following removal of foci of infection and use of vaccine. In most cases the symptoms had existed for a long time and were gradually increasing, despite everything that had been tried previously. Striking examples of improvement following removal of foci and use of vaccine occurred in each of the groups studied, especially where all foci were dealt with. In order to conserve space details of cases will be reported later, elsewhere. Of the thirty-nine patients in whom not all foci were removed and who were vaccinated, 26 per cent improved, 46 per cent remained the same and 28 per cent grew worse. Of the twenty-eight patients in whom not all foci were removed and who were not vaccinated, only 21 per cent improved, 32 per cent remained the same and 47 per cent grew worse. In contrast, of the 216 patients in whom all foci were removed and who received the vaccine, 61 per cent improved, 17 per cent remained the same and 22 per cent grew worse. Of the seventy-five patients in whom all foci were removed but who were not vaccinated, 54 per cent improved, 27 per cent remained the same, and 19 per cent grew worse. Altogether 358 cases were studied. Of this group 149 were encephalitis, mainly of a chronic progressive type, and in only eighteen had symptoms lasted five months or less. There were eighty-one cases of arthritis, most of which were of the progres-

TABLE II

RESULTS FROM REMOVAL OF FOCI OF INFECTION AND USE OF VACCINES PREPARED FROM STREPTOCOCCI THAT HAD ELECTIVE LOCALIZING POWER

VACCINE	ALL FOCI NOT REMOVED				ALL FOCI REMOVED			
	CASES	IMPROVED PER CENT	SAME, PER CENT	WORSE, PER CENT	CASES	IMPROVED PER CENT	SAME, PER CENT	WORSE, PER CENT
Used	39	26	46	28	216	61	17	22
Not used	28	21	32	47	75	54	27	19

sive deformans type. All of the twenty-five cases of spasmodic torticollis were of the idiopathic type without any antecedent history of encephalitis. In sixteen of the seventeen cases of multiple sclerosis symptoms had been present for seven months or more and in eleven of these the symptoms were progressive. There were eleven cases of chronic polyomyelitis and thirteen cases of neuritis or neuro-miositis, in all of which the symptoms had lasted more than six months. Of the twenty cases of ulcer, one was gastric ulcer, nineteen were duodenal ulcer, in six of which gastrojejunal ulcer had developed following gastroenterostomy. Of thirteen cases of lesions of the eyes, seven lesions were progressive, two were stationary, and four were in a period of exacerbation. In all of the fifteen cases of lesions of the skin the symptoms had existed six months or longer. There were fourteen cases of prostatitis. In two cases the condition was acute. In twelve it was chronic.

#### DISCUSSION

The incidence of systemic or metastatic disease in the different groups contrary to what is generally believed by clinicians, was comparatively low following acute infections of the respiratory tract, such as tonsillitis and influenza, and when it did occur there was usually an interval of one to four weeks after the acute attack subsided, a point emphasized in appendicitis by

**Etiology** This is what one should expect for the organisms, usually streptococci, the cause of the acute attacks. They are of high virulence and of high antigenic power, whereas the strains isolated from more or less symptomless foci or from involved tissues, such as of joints, ulcer of the stomach, and in cases of cholecystitis, and appendicitis, are of low general virulence, of lower antigenic power but of high elective localizing power. Hence, the consideration of focal infection in the etiology of disease must go much further than is indicated by the clinical history of acute manifestations in structures that have been shown to be the seat of low grade infections although often symptomless yet teeming with living streptococci that frequently possess elective localizing power.

Owing to the fact that so many of the patients seemed to harbor more than one source of infection it was impossible to determine accurately the relative importance of each. Judging from clinical evidence, however, and the results of cultures and animal experiments it appears to us that localized infection in or around the following tissues predisposes to disease in remote parts of the body in the order named: tonsils, teeth especially pulpless teeth, paranasal sinuses, prostate, cervix, and intestinal tract. Considering the fact that in most instances the disease had existed for a long time in the different groups that it was often progressive and far advanced and that the general measures advised were not much different from those which had been employed previously there would seem to be no doubt but that the relief obtained (Table II) was attributable chiefly to the elimination of foci of infection because it was so marked and in proportion to the thoroughness of removal and, to a lesser degree, to the use of the autogenous vaccine. Improvement occurred with striking regularity when foci were removed in which the causative streptococcus was demonstrated. The fact that exacerbations occurred following removal of foci in some cases also indicates causal relationship and perhaps hypersensitiveness of the affected tissues.

In justice to the patients it was of course impossible to limit the treatment to removal of foci and the use of the vaccine, to the exclusion of various other therapeutic measures. Hence it was not always possible to determine precisely the benefits that were derived from the removal of foci with or without the use of the vaccine and those from the other measures employed.

As has been indicated the patients studied represent in one respect or another unusually difficult cases in each of the groups of diseases and therefore are not comparable with the patients seen as a routine by clinicians. Many of them had not been relieved previously by the use of the usual therapeutic measures such as frequent feedings and allalics in cases of ulcer, local applications in diseases of the skin and physiotherapy, diathermy (usually in sufficiently and intermittently used) diet and orthopedic measures in arthritis, but responded, often in a striking manner when these measures were supplemented by the removal of foci and the use of specific vaccines.

The results especially as regards the importance of focal infection in the cases of ulcer are in general agreement with the clinical observations of Frick, Judd, Eusterman and Rivers and as regards elective localization and vaccine with the experimental studies of Nickel and Hufford, Nakamura, and Meisser.

in ulcer of the stomach and duodenum. In the cases of arthritis the results are in agreement with those of Billings, Nakamura, Moench, and Nickel in chronic arthritis, of Starling in rheumatic infection, and of Rosenow in rheumatic fever. In diseases of the eye the results are in agreement with those of Benedict, Lions and Brown, Haden, Benedict, Von Lackum and Nickel, and Ravdin. In diseases of the skin the results are in agreement with those of Ravitch and Ravitch and Steinberg, and in prostatitis with those of Von Lackum, and Von Lackum and Holloway. After a preliminary survey of cases of encephalitis, Doyle concluded that his information was not sufficient to prove the value of vaccination. Ziegler, in a recent compilation of cases of encephalitis, found that the incidence of improvement in symptoms as measured by the patient's ability to work was not greater in the cases in which vaccine was given than in those in which it was not given. The mortality, however, among the sixty-nine patients vaccinated was only 43 per cent in contrast to 24 per cent of the 125 control patients in this group who were not vaccinated. Ziegler's cases in which vaccine was given, although included in our study, were not subdivided as regards partial or complete removal of focus of infection and therefore his results are not entirely comparable with ours. As would be expected, the results in the encephalitis group, considering the average long duration and the organic changes which no doubt had occurred, were the least favorable of any.

According to the results summarized in Table II, the benefit derived from the removal of focus of infection was increased by only about 7 per cent in the cases in which vaccine was given as compared with cases in which it was not given. This figure is lower than a study of the reports of the cases would indicate, since frequently patients felt better during the period of vaccination, were worse when the vaccine was discontinued, and again improved on resumption of the vaccine. This occurred repeatedly in some cases over a period of several years and certainly was not psychic in origin. We believe that this low average is due in large part to the fact that cases were included in which the chances for relief were well-nigh out of the question, as well as cases in which the attending physician followed the schedule of dosage in cubic centimeters instead of the minimal reaction of the patient. This sometimes led to inadvertent overdosage resulting in nonimprovement or temporary exacerbation of symptoms. Eliminating these cases, the results from vaccine were more favorable than is indicated in Table II. None of the patients, following injection of more than 4200 properly gauged doses of vaccine was made worse permanently. The results from the use of "stock vaccine" made from heterologous strains but true to type and preserved in dense suspensions of glycerol and saturated sodium chloride solution sometimes for many months were seemingly as good as those from freshly prepared autogenous vaccines. Hence, the practicability of the method is assured. The favorable clinical results from the use of these autogenous vaccines are in accord with the fact that specific protection has been obtained in animals against streptococci from ulcer and encephalitis, respectively, through immunization with the corresponding vaccines. Since the streptococci having elective localizing power belong to the viridans type in each of the diseases studied (the type con-

ceded by Holman to possess high invasive power and to occur with great regularity in suspected foci and which in other diseases localize in the tissues of animals and bring about lesions comparable to those in man), since improvement occurred in suitable cases following removal of foci and the use of the specific vaccine made from these streptococci, and since streptococci were demonstrable in the lesions in some of the diseases, the conclusion that the streptococci isolated had etiologic relationship seems warranted. Hence, aside from the benefit afforded patients by the removal of foci of infection and much corroborative evidence of previous work, we regard the ability to isolate the causative organism (usually streptococci from various infection foci in patients suffering from diverse diseases) by the methods outlined as of prime importance, for it makes possible what otherwise would not be possible in many of the diseases studied, attempts at specific prevention and treatment that may reasonably be expected in the near future to do far more good than the heat killed vaccine which we have used. Since streptococci appear to be a cause of so many diseases, since immunity is of short duration, and since mechanical factors play so large a part in maintaining infection in structures so commonly the seat of foci even after prolonged use of specific vaccines mechanical correction or removal so far as possible will probably always be necessary even if highly effective specific or other remedial agents are discovered. Recurrence of the previous condition or new localizations are prone to occur unless the predisposing cause, the focus, is eliminated. Likewise, operation will probably always be indicated in many of the chronic systemic lesions, such as chronic indurated ulcer cholecystitis with gallstones and appendicitis with fecal stones or constricted lumen from previous attacks.

Considerable evidence has been obtained in this study which suggests that tissues in the cases of various chronic diseases particularly the diseased tissues, become hypersensitive to the infecting organisms and their toxic products, as indicated by exacerbations which are particularly prone to occur following too strenuous or incomplete removal of foci of infection and by the reactions which sometimes follow injection of autogenous vaccines, a point stressed by Kolmer. If the dosage of vaccine is gauged so that the local and constitutional reactions are relatively slight the tolerance may often be greatly increased even tenfold and the patient improves a result in accord with that of Wheery and others in bronchial asthma. If the dosage is too large tolerance may not increase but actually diminish reactions may become progressively more marked with each succeeding injection and the patient's condition grow worse. This narrow limit of effective dosage determined more by the reaction in the patient than by the number of bacteria and the use, without bacteriologic diagnosis, of stool vaccines made from bacteria long cultivated in the laboratory have, we believe, contributed much to the general disrepute into which vaccine therapy has unfortunately fallen.

#### CONCLUSIONS

Foci of infection and the elective or specific localizing power of green producing streptococci contained therein appear to be of etiologic significance in the diseases studied.

Either the cautious removal of foci of infection or the use of vaccines prepared from streptococci having elective localizing power, or both, are rational forms of treatment that have been proved of value in our experience and are worthy of further trial. The application of such treatment soon after the onset of disease or even before as a preventive measure, is especially indicated.

## REFERENCES

- 1 Benedict, W L The Character of Iritis Caused by Focal Infection, *Arch Ophth* 1 560 568, 1921
- 2 Benedict, W L, Von Lackum, W H, and Nickel, A C The Pelvic Organs as Foci of Infection in Inflammatory Diseases of the Eye, *Arch Ophth* 56 116 123, 1927
- 3 Billings, Frank Chronic Focal Infection as a Causative Factor in Chronic Arthritis, *J A M A* 61 819 822, 1913
- 4 Doyle, J B Clinical Manifestations and Treatment of Epidemic Encephalitis, *California & West Med* 22 558 562, 1924
- 5 Eusterman, G B Recurrent Ulcer of the Stomach and Duodenum Clinical Notes on Incidence, Diagnosis and Etiology, *Minnesota Med* 6 698 702, 1923
- 6 Evans, J S Epidemiology of Acute Appendicitis in Relation to Acute Nasal and Tonsillar Infections, *Wisconsin M J* 17 91 93, 1918
- 7 Frick, Anders Medical Treatment of Peptic Ulcer Without Alkalis, *J A M A* 82 595 599, 1924
- 8 Goeckerman, W H Lupus Erythematosus as a Systemic Disease, *J A M A* 80 542 547, 1923
- 9 Haden, R L Elective Localization in the Eye of Bacteria from Infected Teeth, *Arch Int Med* 32 828 849, 1923
- 10 Holman, W L Focal Infection and "Elective Localization," a Critical Review, *Arch Path & Lab Med* 5 68 136, 1928
- 11 Irons, E E, and Brown, E V L The Etiology of Iritis, *J A M A* 81 1770 1776, 1923
- 12 Judd, E S Gastric and Duodenal Ulcers, *Wisconsin M J* 24 60 62, 1923
- 13 Kolmer, J A Focal Infection from the Laboratory Standpoint, *J A M A* 87 824 826, 1926
- 14 Meisser, J G Further Studies on Elective Localization of Bacteria from Infected Teeth, *J Am Dent A* 12 554 565, 1925
- 15 Moench, L M The Relationship of Chronic Endocervicitis to Focal Infection, with Special Reference to Chronic Arthritis, *J Lab & Clin Med* 9 289 309, 1924
- 16 Nakamura, T A Study of Focal Infection and Elective Localization in Ulcer of the Stomach and in Arthritis, *Ann Surg* 79 29 43, 1924
- 17 Nickel, A C The Localization in Animals of Bacteria Isolated from Foci of Infection, *J A M A* 87 1117 1122, 1926
- 18 Nickel, A C, and Hufford, A R Elective Localization of Streptococci Isolated from Cases of Peptic Ulcer, *Arch Int Med* 41 210 230, 1928
- 19 Ravdin, B D The Results of Focal Infections as Seen in Ophthalmology and Otolaryngology, *Med Rec* 96 975 976, 1919
- 20 Ravitch, M L Focal Infection in Relation to Certain Dermatoses, *J A M A* 67 430 432, 1916
- 21 Ravitch, M L, and Steinberg, S A Relationship of Focal Infections to Certain Dermatoses, *J A M A* 71 1273 1275, 1918
- 22 Rivers, A B Hemorrhagic Focal Gastroduodenal Lesions, Preliminary Report of Three Cases, *Arch Int Med* 39 564 570, 1927
- 23 Rosenow, E C The Etiology and Specific Treatment of Rheumatism, In *Forscher's Therapeutics of Internal Diseases*, New York, D Appleton and Company 5 509 516, 1914
- 24 Rosenow, E C, and Jackson, G H, Jr Microscopic Demonstration of Bacteria in the Lesions of Epidemic Lethargic Encephalitis, *J Infect Dis* 32 144 152, 1923
- 25 Starling, H S Tonsillar and Rheumatic Infections, *Guy's Hosp Rep* 73 388 408, 1923
- 26 Von Lackum, W H Clinical and Experimental Data on Prostatic Infection, *J Urol* 18 293 306, 1927
- 27 Von Lackum, W H, and Holloway, J K Chronic Prostatitis with Special Reference to Its Focal Aspects, *M J & Rec* 122 23 27, 64 67, 1925
- 28 Wherry, W B Tissue Hydration and Its Relation to Susceptibility and Immunity, as Shown by Skin Tests in Asthma, Chronic Sinusitis and Other Infections, *J Infect Dis* 41 177 189, 1927
- 29 Ziegler, L H Follow up Studies on Persons Who Have Had Encephalitis, *J A M A* 91 138 141, 1925

## PURPURA HEMORRHAGICA REPORT OF THREE CASES\*†

By OSCAR B. HUNTER, M.D., WASHINGTON, D.C.

VERY little is known of the clinical pathology of the purpuric diseases aside from a more or less constant low blood platelet count, prolonged bleeding time, deficient contractility of the clot without any marked delay in the coagulation time.

There are many classifications of purpura, based largely upon its clinical manifestations and the complicating sequelae or the concomitant diseases with which it is associated. Greene<sup>1</sup> states that it exhibits but two constant and invariable symptoms: imperfect thrombus formation and subcutaneous hemorrhages. He groups the different clinical forms as follows:

- 1 Complicating purpuras
- 2 Purpuras with arthritic manifestations
- 3 Simple purpura
- 4 Purpura hemorrhagica
- 5 Purpura fulminans

The first includes those seen occurring in acute infectious processes and chronic diseases with cachexia; the second is associated with joint involvement and is usually subdivided into two types: Peliosis rheumatica or Schonlein's disease with the manifestation of moderate symptoms, and Heubach's purpura with severe symptoms, visceral crises, and acute nephritis.

The simple purpura, purpura hemorrhagica and fulminating purpura seem to be very similar idiopathic exhibitions of the same condition, differing largely in the degree of hemorrhage without associated joint lesions or other complicating or predisposing manifestations. These types are most often designated as the idiopathic purpuras.

The three cases which we have to report appear to belong to this group, being neither typically simple nor extremely severe, but appear to be a moderate form of hemorrhagic purpura (*morbus Weilhofti*) with hemorrhages into the skin and from mucous membranes at varying periods, not alarming or intractable, associated with irregular febrile attacks and no marked constitutional symptoms except those from hemorrhage and anemia.

### CASE REPORTS

CASE 1.—Miss A. B. was a young girl of American parents with irrelevant family history except that her father died of pulmonary tuberculosis. She had the usual diseases of childhood with good recovery and no sequelae. Her general health had been good and her habits normal. In the spring of 1924 when she began to menstruate it was noted that

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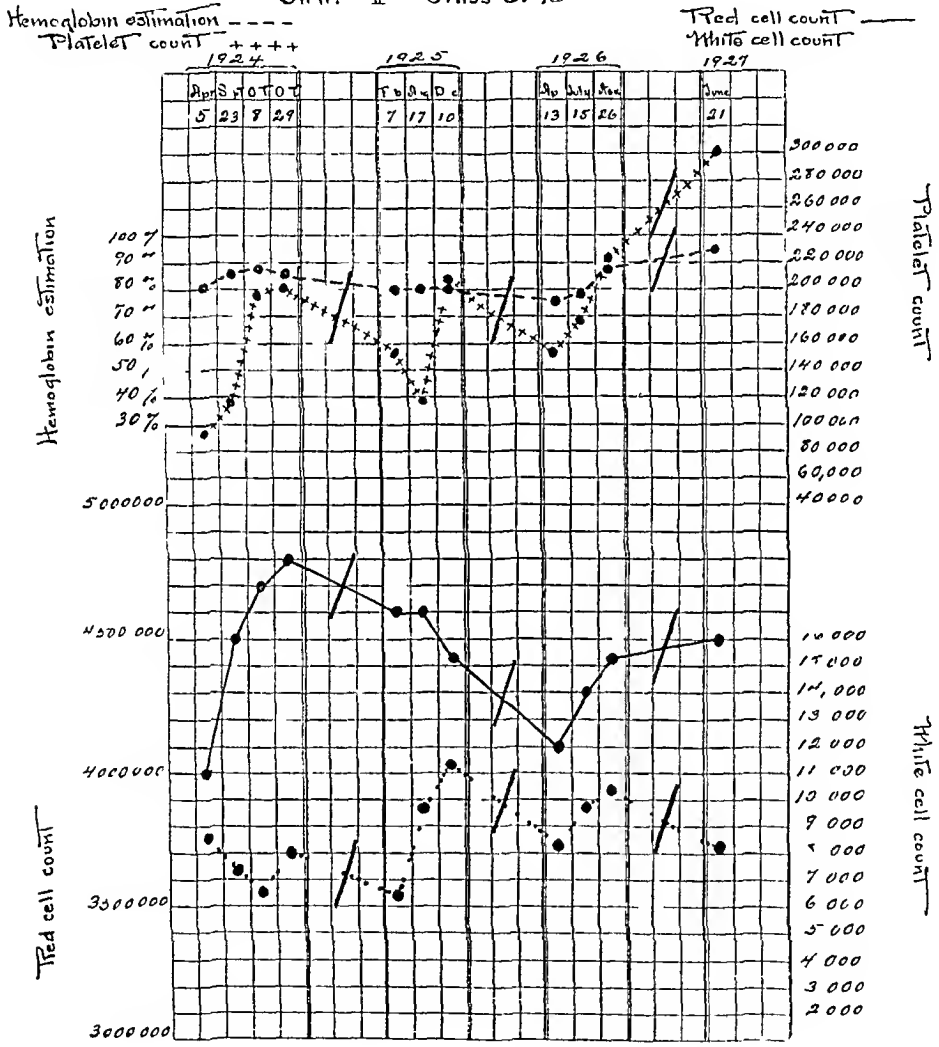
†Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, June 8, 9, and 11, 1928.



purpuric spots appeared, most numerous on her left arm and also to some extent on her thighs, chest and back, this was associated with some bleeding from her gums and rather profuse menstruation

When first seen by us, April 5, 1924, her condition appeared to be that of a simple purpura. She showed a mild degree of anemia (see Table I) with no significant changes in her blood picture other than a reduced platelet count of 92,000. Her coagulation time was normal and bleeding time prolonged to seventeen minutes. Her Wassermann test was negative and her physical condition revealed nothing of a specific or significant character

Chart II Miss A.P.



except a palpable spleen. She was given three transfusions of 500 cc each of whole uncitrated blood, following which she showed marked improvement during the summer and early fall. She was not seen again until February, 1925, when she had a relapse, with irregular response to treatment until December of that year, when she again improved markedly. Following this she was not seen until April, 1926, when she had another relapse, but responded well to transfusion and apparently made a complete recovery.

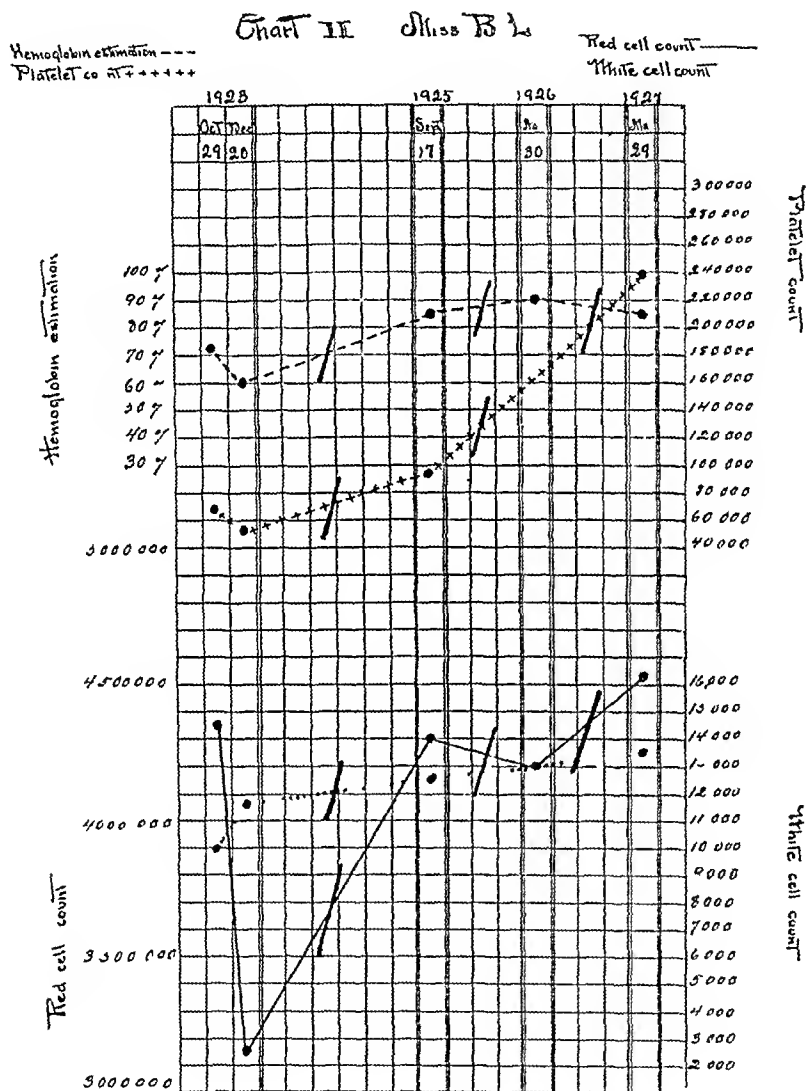
TABLE I  
RECORD OF BLOOD EXAMINATIONS

CASE	DATE	HEMO GLOBIN	ERYTHRO CYTES	PLATE LETS	LEUCO CYTES	DIFFERENTIAL COUNT PER CENT				
						POLYMORPHONUCLEAR NEUTROPHILES	LYMPHOCYTES	LARGE MONONUCLEAR	EOSINOPHILES	BASOPHILES
1	4/ 5/24	80	4 000 000	92 000	4 600	71	23	3	4	
	9/23/24	86	4 500 000	110 000	7 400	73	18	3	4	
	10/ 8/24	88	4 700 000	198 000	6 600	78	24	8	10	
	10/29/24	86	4 800 000	200 000	8 000	53	40	5	1	
	2/ 7/25	80	4 000 000	152 000	6 400	60	34	1	3	
	8/17/25	80	4 000 000	118 000	9 600	71	23	5	1	
	12/10/25	80	4 450 000	210 000	11 400	86	11	1	2	
	4/13/26	76	4 000 000	152 000	8 200	73	23	2	1	
	7/15/26	78	4 200 000	175 000	9 800	78	20	1	1	
	11/ 6/26	88	4 420 000	220 000	10 400	80	15	1	3	1
	1/21/27	85	4 500 000	300 000	8 300	75	20	1	4	
2	10/ 9/ 3	72	4 400 000	64 000	10 000	77	20	1	1	
	12/20/23	60	3 100 000	50 000	11 000	75	17	4	3	1
	9/17/25	80	4 300 000	94 000	12 600	2	20	3	4	1
	11/30/26	00	4 200 000	-	13 000	11	5	3	5	2
	3/29/27	85	4 200 000	23 000	13 600	78	18	1	2	1
3	0/13/27	40	2 430 000	31 000	14 900	61	30	4	2	
	0/10/27	47	2 690 000	73 000	9 500	68	24	3	3	2
	9/24/27	49	2 890 000	138 300	10 100	70	20	1	4	1
	10/ 1/27	74	3 000 000	208 000	12 200	71	20	1	1	1
	10/ 9/27	60	3 830 000	460 000	10 100	75	20	2	2	1
	2/ 1/28	70	4 180 000	330 000	9 200	67	28	3	2	

CASE 2—Miss B L. was a Jewish girl twelve years of age without significant family history, except that her father died of pulmonary hemorrhage of unknown cause. She had had measles, chicken pox, whooping cough and mumps, with good recovery and no sequelae. Her general health had been good and her habits normal except for chronic constipation which was difficult to relieve. During the summer of 1923, she developed purpuric spots scattered more or less over her entire body, bleeding from her gums and blood in the stool with subsequent anemia.

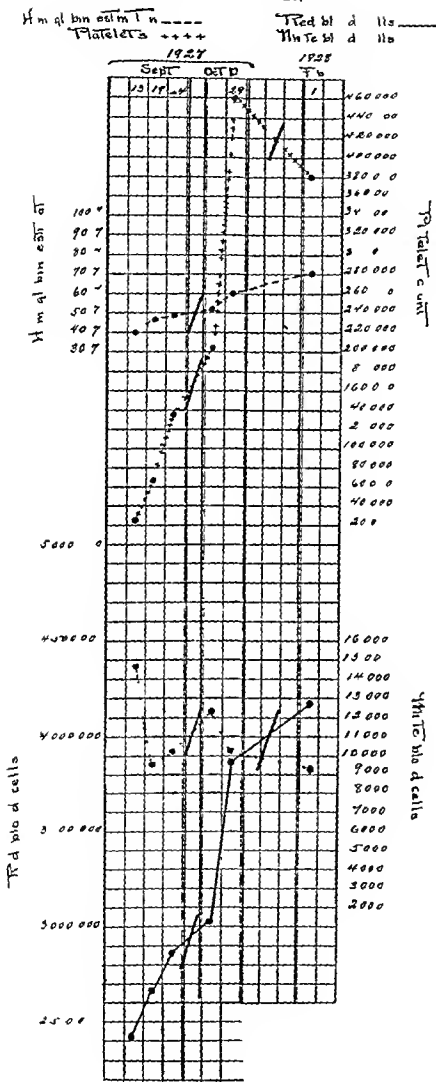
When first seen by us on October 29, 1923, her condition was said to have improved. At this time she was moderately anemic, her blood picture was not especially significant except for the blood platelet count of 68,000 and a prolonged bleeding time of twenty minutes. Her Wassermann test was negative and her physical condition was that of a poorly nourished anemic child with numerous scattered purpuric spots over her body, and spongy bleeding gums. A diagnosis of simple purpura was made. She was not seen again until December of the same year when she had a rather severe relapse, at which time her platelet count had dropped to 50,000 and her erythrocytes to 3,100,000 (see Table). At this time she had a palpable spleen. A diagnosis of purpura hemorrhagica was made and transfusion with splenectomy was advised. This patient was not seen again until September, 1925, shortly after a splenectomy in another city, details concerning which are lacking. Her condition was considerably improved, all bleeding had ceased and she had begun to put on weight. A history was obtained to the effect that she had begun to menstruate during 1924, and had had a very severe relapse from which she nearly died during which time she was transfused. Subsequently her spleen was removed. She thereafter continued to improve until last seen in March, 1927, when her condition appeared to be excellent except for the continued persistently high leucocyte count.

CASE 3—Mrs H M was a white woman, aged twenty two, without significant family history, her father and mother were both living and well. She had the usual diseases of childhood with good recovery and no sequelae. Subcutaneous hemorrhages were first noticed when she was about twelve years old, and appeared usually every summer, as large bluish black bruises without pain. During the summer of 1927 the condition was worse than usual, accompanied by considerable bleeding from the gums and profuse menstruation with marked anemia.



When first seen by us on September 13, 1927, her condition was grave, with a marked anemia (see Table) of 40 per cent hemoglobin and 2,430,000 erythrocytes and a blood platelet count of 31,500. She had numerous hemorrhagic petechiae over her body, bleeding from the gums and profuse metrorrhagia. Her bleeding time was thirty two minutes, coagulation time eight minutes, and her spleen was palpable. She was given two transfusions with considerable improvement in her condition and a splenectomy was done on September 24, 1927. She reacted well from the operation and continued to improve with a decided increase in her hemoglobin, erythrocyte and blood platelet counts. Her leucocyte count was more

## Chart III M. H. M.



or less consistently above the usual normal limits During the month of January, 1928, she was reported to have had bronchopneumonia, from which she made an uneventful recovery When last seen on February 1, 1928, her condition was excellent except for a mild anemia

The spleen removed from this patient measured 15.2 cm long by 10.3 cm broad by 5.6 cm thick, and weighed 322 gm, grossly presenting much of the pathologic picture of the spleen in Banti's disease Its capsule was smooth and glistening and was not adherent, it was relatively firm and turgid, but collapsed to some extent when sectioned The pulp was soft and mushy with engorged sinuses and some interstitial proliferation and stringiness of the reticulum Microscopically there was nothing observed that might be considered characteristic There was a definite increase in the reticulum and considerable proliferation of the endothelium, with an occasional area of focal necrosis in the Malpighian corpuscles There was some increase in phagocytosis and in some instances suggestive blood platelet (?) engulfment

In all three of these cases the outstanding clinical and pathologic manifestations which seemed to be more or less common and characteristic were

- 1 The age of the patients, two were young girls about the age of puberty, and the older patient, a woman of twenty-two years, dated the beginning of her purpura back to about the time of her first menstrual period

- 2 Subcutaneous hemorrhages of a typical purpuric character, scattered more or less over the entire body, without pain, visceral crises or other complicating diseases

- 3 Hemorrhages from the mucous membranes, especially the gums and uterus, particularly at the time of menstruation

- 4 Low blood platelet counts, but not excessively decreased

- 5 Prolonged bleeding time, deficiency in the retraction of the clot without any significant change in the coagulation time

- 6 No significant change in the blood picture, except that of a secondary anemia, with a tendency to a leucocytosis and an increase in the mononuclear and eosinophilic elements

- 7 Sufficient enlargement of the spleen to be definitely palpable upon physical examination

- 8 The definite improvement by transfusion Two were apparently cured by splenectomy

#### REFERENCE

Greene, C. L. Purpura. In Tice, Frederick. Practice of Medicine, Hagerstown, W. F. Prior Co., 1923, No. 6, p. 859

#### DISCUSSION

Dr. John W. Gray—I should like to mention the case of a young child suffering with acute purpura whose life was saved by two transfusions Within a week following the attack two other children in the family developed scarlet fever In this case it would seem that this was an individual reaction on the part of the patient Ordinarily we do not find such reduction in the blood platelets in this type of streptococcus infection

# IMPROVEMENT IN TECHNIC AND RESULTS MADE IN EXAMINING MICROSCOPICALLY BY THE RAZOR SECTION METHOD 2000 MALIGNANT TISSUES\*

By BENJAMIN TAYLOR TERRY, M D ROCHESTER MINNESOTA

**INTRODUCTION**—This is a progress report. My previous publication<sup>1</sup> was based on 600 malignant tissues that had been examined perfectly fresh without any fixation whatsoever. The present report is on 2000 malignant tissues. In this 2000 are included many extremely minute specimens on which a year ago I would have failed. The handling of these tiny bits of tissue has necessitated modifications in the technic. The improvements worked out on minute bits of tissue, make it possible to stain the larger specimens more uniformly and successfully. The changes in technic and in the results of the examinations, will be described in this paper.

**Advantages**—The advantages pointed out in my previous paper<sup>1</sup> are present in the improved technic. My method is quickly learned, is extremely rapid, uses no carbon dioxide, is inexpensive, noiseless, dependable and can effect a great saving of time for the surgeon, pathologist, and patient. Moreover, the equipment is simple, light and so compact that it is readily transported. This makes it possible to employ the method in the operating room as Christeller<sup>2</sup> is now doing. The method will probably continue to be most useful in the rapid microscopic examination of malignant tissues, but it shows also inflammatory and other pathologic changes. It is of great value in selecting blocks to be examined by other methods. It permits the exact orientation of each section and makes the pathologist largely independent of a technician. This last point is of special importance when emergency examinations have to be made. Moreover, microscopic examination by the razor section method does not interfere with the subsequent examination of the same tissue by other methods. Artefacts are few for tissues can be examined in which the cells are seemingly still alive. If appearances regarded as artefacts in other methods are observed also in razor sections of perfectly fresh tissue, they are probably not artefacts.

**Disadvantages**—The razor section method has three disadvantages. (1) If the tissue is not fixed, the sections are not permanent. In this respect razor sections resemble frozen sections of fresh unfixed tissue. To secure permanent specimens it is necessary to fix the tissue and to employ some other stain, e.g., hematoxylin. (2) The cells which are beautifully stained at first fade quickly. It is necessary, therefore, to examine the sections immediately after staining; the sooner they are seen the more satisfactory they are. But faded sections can be repeatedly restained. (3) The razor section method while suitable for

Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 1928.

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Safety razor can be easily secured from any good barber. Safety razor blades and dermatome knives can also be employed and the Antioch Utility Knife has been found useful and inexpensive. Best of all, however, in my experience is the old fashioned bone razor still used by most barbers.

The best cutting board I have yet found is a piece of compressed cork. A rubber mat made of this material is excellent. Balsa wood also makes a good cutting board. The advantages of this very light wood were brought to my attention by Lieutenant Commander Rigle of the U.S.S. Hospital Ship Relief.

**The Stain**—The stain employed must be good if the results are to be satisfactory. Neutralized polychrome methylene blue made according to the directions given in my paper<sup>6</sup> a year ago is the best stain I have yet tried. It works with astonishing rapidity on both fresh and fixed tissue. It is also effective on tissues which have been rapidly fixed in formalin although these frequently do not stain as well as unfixed tissue or tissue which has been thoroughly fixed. Neutralized polychrome methylene blue can be evaporated to dryness without injury and can be preserved as a powder. It goes instantly into solution when distilled water is added and is at once ready to use.

The best light I have tried is a 60 watt white Mazda bulb which has been frosted. This bulb should be supported and shaded. By keeping the lamp four or more inches from the microscope its heat is no longer objectionable.

Slides with holes 2 cm. in diameter cut entirely through them can be easily made out of any soft wood. They are especially convenient for mounting sections which are not of uniform thickness.

It is desirable to keep the razor blade clean and smooth as well as sharp. Wipe the blade off immediately after using. If it is rusted or has clotted blood or bits of dried tissue clinging to it the surface of the tissue will be scratched and the microscopic picture will not be satisfactory.

#### TECHNICAL STEPS

1 **Selection**—When malignant changes are looked for it is important that the right area be selected for examination. If this is not done, the diagnosis will be missed. To select the best area the pathologist should be a good diag-

##### Neutralized Polychrome Methylene Blue

This stain keeps well and acts rapidly, precisely and differentially on both perfectly fresh unfixed tissue and on formalin fixed tissue. There are five steps in making it. (1) The preparation of stock solutions A, B and C. (2) The titration of Solution A against Solution C. (3) The alkalization of B by means of A. (4) The polychroming of the alkalized B. (5) The neutralization by means of C of alkali already added to B.

The first five steps in detail are as follows:

1. The three stock solutions in neutral distilled water are

A	1% $\text{H}_2\text{CO}_3$ C.P. anhydrous	100 C.C.
B	1% methylene blue medicinal	1000 C.C.
C	10% acetic acid by volume	100 C.C.

**Titration**—Determine how much of Solution A exactly neutralizes 1 c.c. of boiling standard Solution C using phenolphthalein as an indicator. Mark this quantity on Bottle A.

3 **Alkalization**—Into a 100 c.c. graduate place that quantity of A which is equivalent to 1 c.c. of C add enough of B to make 100 c.c. and mix thoroughly.

4 **Polychroming**—Of the alkalized methylene blue 5 c.c. are placed in each of four one ounce bottles. These are food unstopped in cold water which is brought to a boil in about 10 minutes. Note the time and remove the bottles one by one 15, 30, and 45 minutes later. Let them cool slowly. The water after reaching the boiling point should be kept boiling while the bottles are in it.

5 **Neutralization**—To each 5 c.c. of the polychrome stain add 0.5 c.c. of Solution C. Filtration is usually unnecessary and should not be carried out immediately. The four bottles are polychromed differently to permit each worker to make and choose stains which best suit his own taste, light and work.

Two firms in New York City, the National Aniline and Chemical Company, 40 Rector Street, and Elmer and Amend Third Avenue at 18th Street have begun to make the stain here described and each has agreed to submit samples for my approval before marketing it.



nostician both of the gross and the microscopic changes. Malignant tissues usually depart from the normal. They may be softer, or harder, or more friable. They may be more pink and they are frequently somewhat yellow or contain yellowish specks. By palpation, section, and inspection the areas suspicious of malignancy are selected. From the entire unfixed specimen the pathologist should be permitted to choose for his examination the areas which in his judgment are most likely to yield the evidence sought. The results are apt to be less satisfactory when the surgeon excises the tissue for the pathologist and sends it to him in a bottle of fixative, for after fixation, tissue no longer has its original color or consistence and it is frequently distorted. It becomes often a matter of great difficulty to know how to section such tissue so as to obtain the best results. When tissues are fresh, it is usually easy to

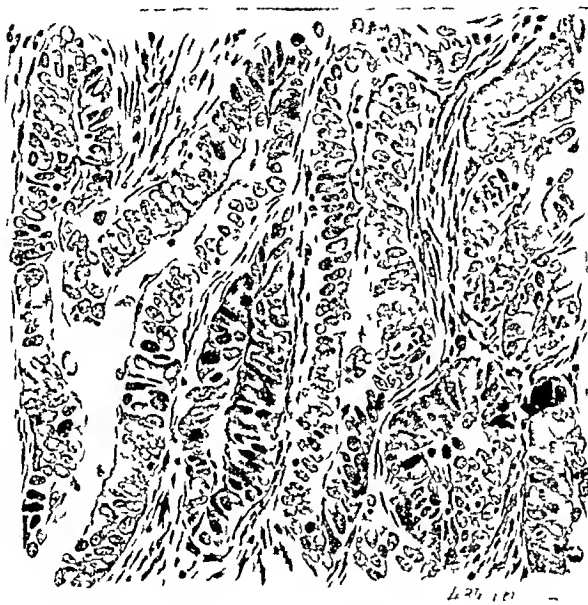


Fig 1—Razor section of an adenocarcinoma of the stomach. Stained by N P M B (Grade 3 Broders)

detect by palpation lymph nodes in which carcinomatous metastases of large size are growing, but after large masses of tissue have been thoroughly fixed in formalin it may take hours to locate a small metastasis that in the fresh state might have been found in a few minutes.

**2 Excision**—The area selected by the pathologist should, if possible, include the advancing edge of the tumor. Before this area is cut out the next two technical steps must be carefully considered, namely, the immobilization and the slicing of the tissue. The tissue should be so excised as to make both of these steps as easy and as satisfactory as possible. It is important to realize that the block removed for razor section should be thicker than that taken for frozen sections. In the case of frozen sections the tissue is usually thin and flat and the broadest surface is frozen to a table. In sectioning this the microtome knife cuts in a direction parallel to the surface of the table. For razor

sections the broadest and flattest surface is pinned to the cork cutting board but the razor blade in sectioning is employed perpendicular to the surface of the cork. This means that the tissue to be cut by the razor should be taken differently from that to be sectioned by the freezing microtome. If the surgeon fails to think of this he usually gives the pathologist sections which are unnecessarily hard to cut and diagnose. A rectangular block measuring  $1 \times 1 \times 0.5$  cm is easily sectioned by the razor but larger or smaller blocks may also be cut. A small block of tissue properly selected is much easier to diagnose than a larger one which is not so carefully chosen.

3 *Rapid Fixation*—The rapid fixation of tissue in hot formalin is usually not necessary, but at times it is a great aid especially if the tissues are so soft that sectioning is quite difficult. Large amounts of colloid or mucus in

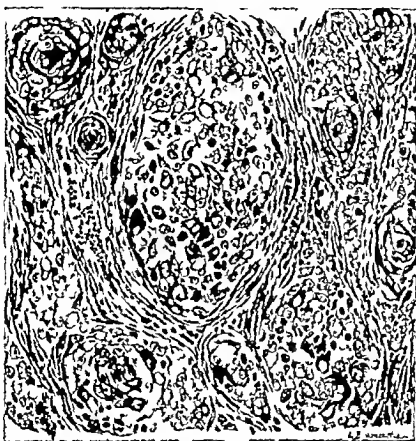


Fig 2—Razor section of squamous cell carcinoma of the pharynx. Stained by N P M B (Grade 3 Broders)

unfixed tissue occasionally make the subsequent staining unsatisfactory. This is due to the fact that mucus and colloid ooze up on the cut surface and so cover the cells that the stain when applied does not reach the cells that should be stained, such tissues are easy to examine after fixation. If difficulty is experienced in sectioning fresh tissue it is advisable to drop small blocks of this into bottles containing formalin heated from  $95$  to  $98^{\circ}\text{C}$ . At this temperature small pieces of tissue are quickly coagulated and usually in sixty seconds the consistency is much more suitable for thin sectioning. Fixation is also of advantage where the tissues are deeply pigmented or red.

4 *Immobilization*—Tissue must not move while it is being cut. The block should have at least one flat surface. The flat surface is pressed gently against the cork cutting board and two or more pins are thrust through the tissue fastening it securely to the board. For one who is right handed it is conven-

ient to pin the tissue 2 or 3 cm above and to the left of the lower right corner of the cutting board. If the block consists of soft tissue to which is attached firmer tissue or a dense capsule, the tougher or firmer parts if flat should be placed next to the cutting board. If they are not flat they should be so pinned that they are cut after the razor has passed through the softer portions. If this is not done the softer tissue may be crushed.

5 *Wetting*—Both the tissue and the razor should be wet before the tissue is sliced. Distilled water or clear cool tap water may be employed. By wetting the tissue and razor, friction is reduced and sectioning is made easier and more satisfactory.

6 *Slicing*—The object is to obtain one or more thin, smooth, plane-parallel slices which shall contain the area that one wishes to examine micro-

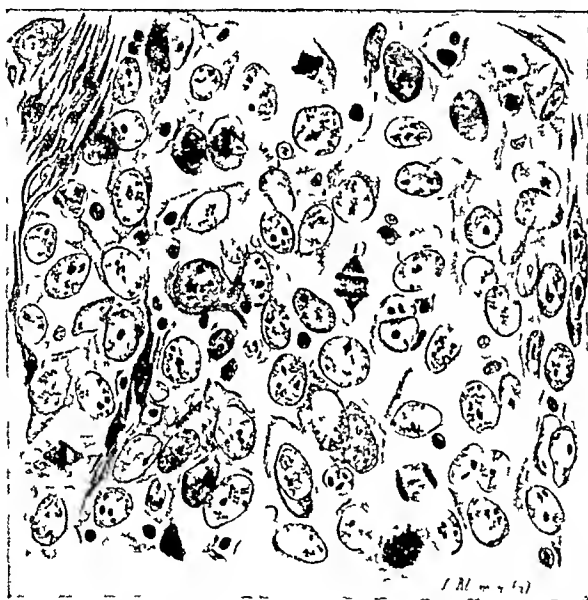


Fig 3—Razor section of a solid carcinoma of the uterus. Stained by N P M B (Grade 4 Broders)

scopically. The point of the razor rests on the cork cutting board 5 to 6 cm to the left of the immobilized block. If properly placed, the point is not raised again until the section is completely severed from the block. Before sectioning, the other end of the razor blade is raised and then lowered over the block. By sighting over the back of the razor, one can see how much tissue will be cut off. The tissue may be further supported by sticking forceps in the cutting board in line with the pins and against the right side of the block. By moving the upper part of the forceps forward or backward, they can be made to support the back of the razor blade. This support is a great aid if the hand of the operator is unsteady. In cutting the section the razor is neither raised nor lowered but is drawn along the cork so that the full length of the blade passes through the tissue at each stroke. The first cut is made 1 to 2 mm in front of and parallel to the supporting pins. If the

razor is very sharp its own weight as it is drawn along will be almost enough to cause it to cut entirely through tissue of average resistance Unless the tissue is very limited in amount the first piece cut off is discarded Without changing the position of the razor in the hand, place the point once more in the cork to the left of the immobilized block and sighting over the back of the blade see how thin a piece you can cut by drawing the wet razor again through the tissue In this way secure a thin, plane parallel slice which can be stained at once In slicing be careful not to bring the edge of the razor in contact with the pins

With practice surprisingly thin sections can be cut with a razor but thin sectioning can be overdone If the sections are too thin, they are harder to handle and before staining are best mounted on slides drawing the slide and the section together out of a glass of water This usually causes the section to lie flat on the slide Sections which you are apt to regard as too thick, often give excellent results If the amount of tissue is small, every section cut should be stained and examined

*Slicing Thin Blocks*—This technique is new and learning it has enabled me to make diagnoses which before were impossible The cutting board is first moved to the edge of the table so that the hand in cutting will not be hampered in any way If you are right handed the tissue is placed on the lower right corner of the cutting board and is held against it lightly but securely by a small cork stopper the small end being down and so resting on the board that the tissue is prevented from slipping toward the operator as he cuts it Both the tissue and the razor blade are wet The razor is placed flat on the cutting board and is brought close to the tissue The back of the razor blade remains in contact with the cutting board but the edge is raised slightly until one can see a small space separating the board from the edge of the blade The razor is now drawn through the tissue using a single long stroke in which the entire length of the blade is employed The result is often surprisingly good At times I have been able to make three or four sections out of one slice that seemed already too thin to section further Moreover, if the cutting is skillfully done and if little pressure is exerted on the tissue, smooth surfaces are made with each stroke of the razor

7 *Staining*—The soundness of the practice of applying stain superficially to one side only of a slice of tissue has been abundantly confirmed by the large number of sections examined in the last year But the method has been improved The former method which consisted in drawing a section with forceps over a thin film of stain, had several disadvantages

- 1 The surface stained is not seen until after it is stained Irregularities in staining are therefore hard to avoid

- 2 The method becomes less and less satisfactory the smaller or narrower the section, for it is difficult with the old method to prevent these sections from being stained on both sides

- 3 The portion of tissue grasped by the forceps remains unstained Occasionally the very area that should be studied carefully may in this way escape observation

4 Some tissues are so friable that thin sections cannot be pulled along without developing cracks into which the stain runs. Sometimes the sections have broken in two.

The problem of how to alter the technic so as to stain successfully sections that are unsatisfactory with the old technic has apparently been solved. Instead of dragging tissue over a thin film of stain, the tissue is now mounted with the side to be stained uppermost. *This side must be smooth.* Beneath the section water is allowed to run so that every crevice is filled. Thus I refer to as "water sealing," for it prevents stain applied to the upper surface from running beneath and staining the under surface of the section.

*Staining Minute Specimens*—The smaller the surface, the smaller the quantity of stain to be used. The surface to be stained should be smooth and moist, but not covered with a thick film of water. If stain is added to a section on which a drop of water is standing, the stain immediately diffuses over the surface of the drop and into the upper layers of the water without reaching the section. Excess of water on the surface should, therefore, be removed by a medicine dropper or by a small piece of smooth filter paper. If the section is extremely small all operations are best carried out under a dissecting microscope and the stain is diluted somewhat before it is applied to the surface by a small pointed camel's hair brush, or by a dissecting needle the point of which has been flattened by rubbing on a hone. The needle is dipped into a thin film of stain and carries over only a very small fraction of a drop when it is applied to the surface of the tissue. The needle has the further advantage that tiny bits of tissue are not picked up by it, as sometimes happens when a camel's hair brush is used. As soon as the stain is applied it is spread quickly over the surface, care being taken not to scratch or injure this surface. The section is then immediately washed with water to prevent the stain from sinking into the depths. By this technic bits of tissue not larger than a pin's head have frequently been beautifully stained.

*Staining Frozen Section Remnants*—By using the technic just described I have frequently been able to stain without further section tiny bits of tissue, such as minute carcinomas of the vocal cords, that had already been examined on the freezing microtome. One side of these remnants is smooth and the other rough. All that was necessary for success was to pick out under the dissecting microscope the smooth surface and to stain this.

*Staining Larger Specimens*—The technic worked out for minute specimens makes the staining of larger ones easy and frequently more satisfactory than it was before. The more superficial the staining, the more brilliant the result. The section is mounted on a glass slide smooth side uppermost and water is allowed to run under the specimen to fill completely all crevices. The upper surface is drained of its water and the stain is applied with a small camel's hair brush. If the available tissue is limited to one section, the stain may be diluted to avoid danger of overstaining. It is at times advantageous to stain even the larger specimens under a dissecting microscope.

8 *Washing*—As soon as the upper surface has taken up the stain, it is immediately washed with a large quantity of water. It is convenient to have

a medicine dropper filled with tap water in the left hand while the stain is being applied to the surface of the tissue with the right. Immediately after staining, water is spouted under, then over the stained section. It is a mistake to apply only a small quantity of water to a large section on which an excess of stain has been placed. Under these conditions the stain is merely diluted and may be forced under the section and stain it.

9 *Mounting*—Nothing new has been learned about mounting the sections. There are two methods. If the section is thin and plane parallel the stained surface is placed in contact with the cover glass the cover is turned over, and is then mounted on a glass slide on which a drop of clear water has already been placed. The second method is to be employed when the section is thicker at one end than at the other. The section is mounted on a cover as before, but this cover instead of being placed on an ordinary glass slide, is turned over and mounted on a slide in the center of which a hole has been cut. The tissue lies in this hole.

10 *Examining*—It is important to examine the stained specimen at once by transmitted light. If this is not done the staining will appear unsatisfactory, for the sections fade rapidly. The light should be strong enough to transilluminate the tissue but it is well to avoid using more light than is needed. It is best to cut the diaphragm down until only a small illuminated circle about 3 mm. in diameter is seen where the light strikes the tissue. The intensity of the light may also be regulated by moving the lamp or the mirror or by lowering or raising the condenser of the microscope. If the section fades before the examination is complete it can be restained one or more times. But the restaining cannot be repeated indefinitely without decolorization, as the stain gradually sinks into the depths and then alters or absorbs so much light that the specimen is no longer properly illuminated. Stained sections can be completely decolorized by placing them in alcohol or in a large volume of formalin. After decolorization sections can be preserved in formalin and restained whenever it is desired.

*Diagnosing*—The rapid microscopic diagnosis of razor sections is often easy, but it is sometimes difficult. The harder the diagnosis, the more one should know about the tissue and the better the technic should be. Before attempting the microscopic diagnosis the pathologist should examine the tissue in the gross. It is far more important to know the exact source of the specimen, its size, shape, color and consistency than it is to know the history of the case. Razor sections of fresh unfixed tissue as well as of fixed tissue if stained with neutralized polychrome methylene blue resemble good frozen sections stained with the same stain. Those pathologists who are familiar with the staining reaction of polychrome methylene blue on frozen sections should have no difficulty with razor sections similarly stained. But pathologists who have had no experience with polychrome methylene blue should always control their razor section diagnoses by the method they know best.

*Hematoxylin*—The nuclear stain most used by pathologists is hematoxylin. Many of those who have used this dye for years and are thoroughly familiar with it do not feel competent to diagnose sections stained with polychrome

methylene blue For this reason and also because hematoxylin is not extracted from the sections in the process of clearing to make permanent preparations, it seemed desirable to test this stain Although my experiments are not numerous, they are encouraging Razor sections of fixed tissue can often be stained with hematoxylin and be ready for examination in less than sixty seconds If the section is understained, it can easily be restained In applying the stain try to keep it from penetrating deeply or staining both sides of the section To prevent the diffusion of the stain into the depths, razor sections before staining are blotted lightly on smooth filter paper A thin film of hematoxylin is then obtained by adding a drop or two of the stain to a small piece of filter paper on a glass slide The section is placed on this film and the stain is allowed to act for that length of time which experience shows is necessary With the sample of Harris' hematoxylin that I use, thirty to sixty seconds usually suffice, although occasionally better results are obtained with a somewhat longer application The sections stained with hematoxylin do not show the contrast given by neutralized polychrome methylene blue, but nuclear detail and mitotic figures can be brought out with great sharpness and for purposes of diagnosis these sections are quite satisfactory They have one very great advantage They do not fade quickly They hold their stain well for days or weeks and possibly longer For those who employ hematoxylin as a routine and who are not familiar with the results obtained by staining sections with neutralized polychrome methylene blue, the sections stained with hematoxylin are often very satisfactory

In Table I are recorded the results obtained in 1926-28 in examining 600 malignant tissues The results obtained on fresh unfixed tissue are shown in column 2 In the third column are the results obtained after applying the improved technique described in this paper to the tissues which gave me difficulty when first examined These early records are not fair to the method, for in the first 200 examinations I was often much disturbed by visitors who came to see the method and who took my attention when it should have been given to recording my findings If I did not write down at the time of examination that the diagnosis was easily obtained by me, the result was subsequently recorded as a failure Some of these failures were merely omissions In my previous paper only the results of examining perfectly fresh tissue were reported I could not, therefore, include any of my examinations of fixed tissue But now that my technique is applicable to both fresh and fixed tissue, it has seemed desirable for me to reexamine those tissues which either

TABLE I

MALIGNANT TISSUES EXAMINED	NUMBER OF CASES OF AGREE- MENT WHEN MY EXAMI- NATIONS WERE LIMITED TO FRESH UNFIXED TISSUE	NUMBER OF CASES OF AGREE- MENT UPON REEXAMINA- TION OF THE DIFFI- CULT CASES AFTER FIXATION
1-100	62	99
101-200	82	97
201-300	91	98
301-400	97	98
401-500	92	98
501-600	96	100

gave me difficulty or on which no record of the success of the examination had been made. This has been done and the results are shown in Table I.

In Table II it is seen that in about 98 per cent of the cases, my razor section diagnosis check satisfactorily with those made by the pathologists of the Mayo Clinic using microtomes. But between the 1701 examination and the 1800 the results check in only 94 per cent of the cases. An explanation may be of interest. The tissues which I receive for examination have at times passed through the hands of a number of men before they reach me. Occasionally as a result of repeated examinations of one small area of malignancy, little or none of it remains for me to find. The six tissues between the 1701 and 1800 examination in which I was unable to confirm the diagnosis of

TABLE II

MALIGNANT TISSUES EXAMINED	NUMBER OF INSTANCES IN WHICH MY DIAGNOSES MADE ON RAZOR SECTIONS CHECKED SATIS- FACTORILY WITH THOSE MADE BY MAYO CLINIC PATHOLOGISTS ON FROZEN SECTIONS		MALIGNANT TISSUES EXAMINED	NUMBER OF INSTANCES IN WHICH MY DIAGNOSES MADE ON RAZOR SECTIONS CHECKED SATIS- FACTORILY WITH THOSE MADE BY MAYO CLINIC PATHOLOGISTS ON FROZEN SECTIONS	
601 700		98	1301 1400		98
701 800		98	1401 1500		100
801 900		100	1501 1600		98
901 1000		99	1601 1700		98
1001 1100		97	1701 1800		94
1101 1200		99	1801 1900		98
1201 1300		97	1901 2000		99

malignancy were returned to the hospital from which they came originally and were carefully reexamined by one of the pathologists, Dr. Wellbrock. In only one of these six cases was Dr. Wellbrock able to find any evidence of malignancy and in this instance the malignant changes were so minute that they were found in one section only and even then they were so inconspicuous that it was extremely difficult to be sure that they were present at all. My percentage of agreement therefore in this 100 although recorded as 94 when compared with the original diagnoses, would be 99 per cent as checked by Dr. Wellbrock when he examined the same material that I examined.

*Dependability*—Repeatedly in examining perfectly fresh tissues diagnoses have been reported to the operating room by Drs. MacCarty, Broders, and Caylor on razor sections before the frozen sections were ready to be examined. Dr. William Carpenter MacCarty when he discussed my paper at the last meeting of this Society said, "I would be perfectly willing to take a bottle of his stain, a couple of pins and a razor and make almost any diagnosis that I would make by any other method. I do not need the microtome."

So far as actual recognition of the things which are diagnostic I would as soon have Dr. Terry's preparation as any made with the freezing microtome. Razor sections have been good enough for Dr. MacCarty's<sup>4</sup> to make from them his cytologic diagnosis of malignancy and they have enabled Dr. Broders<sup>7</sup> to apply successfully his rules for grading malignancy.

*Microdrawings*—The three microdrawings with which this paper is illustrated were made from razor sections stained with neutralized polychrome



methylene blue For them I am indebted to Dr Erwin Christeller who not only selected from his own material the areas to be drawn but at every step controlled the accuracy of the reproductions made by his artist These drawings show, as no words can do, the great nuclear detail that can be seen if the razor section technic is properly carried out The original drawings in colors are so much like the microscopic specimens that from them alone Dr A C Bidders was able without difficulty to grade the malignancy of each of these tumors

*Tested by Others*—The razor section method has now been tested by many physicians When they have carried out the technic under my supervision and on favorable tissues, no one has failed Most doctors make good sections at the first or second attempt Christeller<sup>2</sup> has recently published a very interesting article in which he describes his experiences with the method He frequently is called upon by surgeons to make immediate microscopic examinations As most of his diagnoses are secured in thirty to forty seconds, he says the surgeon loses no time as it takes him this long to stop bleeding In 104 razor section examinations by Christeller<sup>2</sup> the diagnoses thus made checked satisfactorily with those obtained by other methods in 101 instances

#### SUMMARY

The razor section method here described has many advantages It is rapid, easy, inexpensive, and dependable, and it is applicable both to fresh unfixed tissue and to tissue fixed in formalin No carbon dioxide is employed Artefacts are few In studying by the razor section method 2000 malignant tissues a number of improvements in technic have been made These permit the examination of tissues on which I would have failed a year ago Thin slices of tissue can be cut still thinner and very minute specimens can now be successfully stained and mounted The rapid fixation of some tissues in hot formalin has been found advantageous, and razor sections which do not fade quickly have been secured by staining with hematoxylin By using the improved technic and restudying tissues that have given difficulty the diagnoses made on razor sections of 2000 tissues have agreed satisfactorily with those obtained by the pathologists of the Mayo Clinic using microtomes in 98 per cent of the cases

#### CONCLUSION

The new technic has so many advantages that it seems that every pathologist should be willing to give it a fair trial But no claim of perfection is made The razor section method should not replace other methods Instead, it should be used in conjunction with them

*Acknowledgments*—During the last two years I have been privileged to work as a guest in the Department of Surgical Pathology of the Mayo Clinic Free access has been given me to an abundance of perfectly fresh as well as fixed malignant material For the splendid spirit of helpful cooperation shown me I am deeply grateful and wish to express here my cordial thanks to all who have assisted me I am especially indebted to Drs McCarty, Bidders, Caylor, Wellbrock, Robertson, and Mills

## REFERENCES

- 1 Terry, B T A New and Rapid Method of Examining Tissue Microscopically for Malignancy, *J LAB & CLIN MED* 13 550 563 1928
- 2 Christeller, Erwin Erfahrungen mit der Verbesserten Histologisch Diagnostischen Schnell Methode nach Terry, *Klin Wchnschr* 7 448-481 1928
- 3 MacCarty W C The Cytologic Diagnosis of Neoplasms, *J A M A* 81 519 522 1923
- 4 MacCarty, W C Early Diagnosis of Cancer *Arch Clin Cancer Res* 1 11 20 1925
- 5 MacCarty W C The Cancer Cell and Nature's Defensive Mechanism *Surg Gynee Obst* 41 783 793 1925
- 6 Broders, A Squamous Cell Epithelioma of the Lip, *J A M A* 74 656 664 1920
- 7 Broders A C Cancer's Self-control, *Med J & Rec* 211 133 135, 1920

## VALUE OF LEUCOCYTE COUNTS, ACCORDING TO SCHILLING FORMULA IN CLINICAL MEDICINE\*

By F W NIEHAUS M D OMAHA

THE numerical and the differential leucocyte count is recognized as a valuable laboratory procedure, especially in infections it is regarded as an index of severity, and frequently is an important factor in deciding whether or not to perform a major surgical operation. But this measure is not always infallible and often the leucocyte count does not furnish the anticipated information.

The method to be considered here has been found to minimize greatly these disappointments.

The first attempt to modify the conventional leucocyte count was made by Arneth in 1904 the chief point of which was a classification of the neutrophils according to the nuclear morphology. His method was rather intricate, and too ponderous and time consuming for use in clinical medicine.

Cooke<sup>1</sup> modified this method dividing the neutrophils into five groups. This is a practical method and furnishes as much information as the more complicated Arneth classification, and is still used by Ponder.

Schilling<sup>2</sup> recently modified Arneth's method. His method is much simpler, in fact, with good preparations it does not appreciably increase the time and labor of an ordinary differential, and markedly adds to its value. His classification (Fig 1) of leucocytes is as follows:

1 *Myelocytes*—The nucleus is round, oval, or kidney shaped. It is relatively large, vesicular and stains palely. It is coarsely granular, and usually has a nucleolus. The cytoplasm is pale blue. The granulations are usually delicate, and stain weakly. These are normally found in the bone marrow but never in the peripheral blood.

2 *Young Forms*—Normally many are present in the bone marrow, rarely in the peripheral blood. The nucleus is sausage or bean shaped. It is vesicu-

\*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists Minneapolis Minn June 8, 9 and 11 1924

lar, and does not stain intensely. There are granulations in the ends of the nucleus. The cytoplasm is like mature cells. At times it is distinctly light blue, the granulations are not so distinct. The cells are usually slightly larger than mature cells. This class is, in part, identical with Pappenheim's "Metamyelocytes."

**3 Staff Forms**—The nucleus is T, U, or V shaped. The cytoplasm is fully mature. These cells constitute 3 to 5 per cent of the leucocytes of normal blood.

Degenerative staff forms are apparently mature neutrophils without segmentation. On account of a developmental inhibition, the sausage form does not divide into segments. They are differentiated from the normal staff forms and the young forms by small band-like, often bizarre, twisted and always

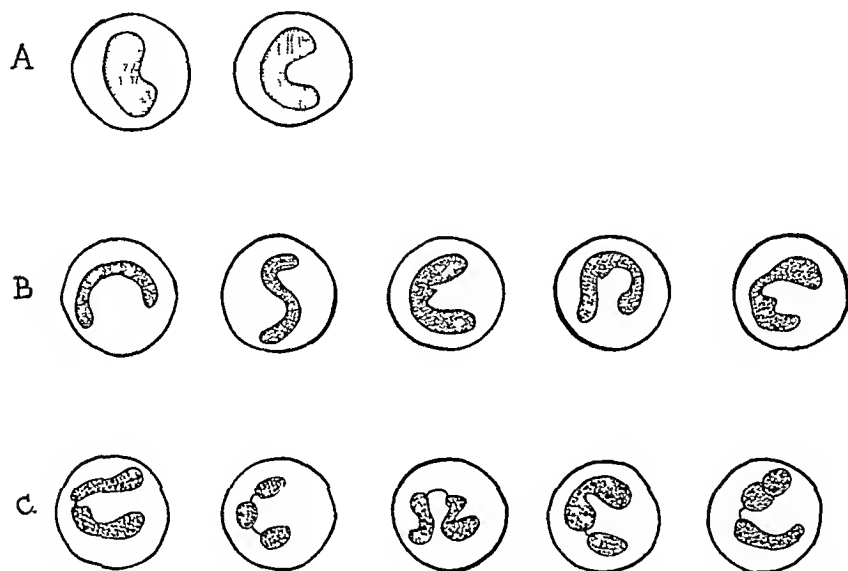


Fig. 1—Nuclear morphology of neutrophils. Group A young forms. Group B staff forms. Group C segmented forms.

hyperchromatic (dark, structureless) nuclear form. The granulations either easily overstain or stain feebly and are partially dissolved. These easily

described as at least twice the size of a red blood cell, usually much larger than a granulocyte. The cytoplasm is relatively wide, stains smoky blue to pale violet and it frequently contains small vacuoles. The nucleus is of a medium size, or relatively large. Its shape is oval or bean shaped, it is placed slightly eccentric, the wall is never entirely smooth. It may even be sausage shaped, or have plump segments.

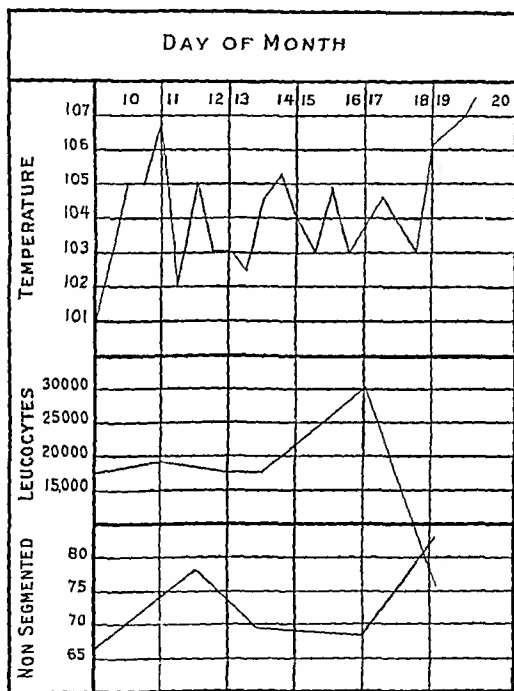


Chart 1

Schilling further conceives the origin of white corpuscles from three sources (Triasismus) namely. The granulocytes from the bone marrow, the lymphocytes from lymph glands and lymphoid tissue wherever found, the monocytes from the reticulo endothelial tissue. The peripheral blood picture reflects the functional status of these organs or tissues, as influenced by their disease or by their response to exciting agents as infection. This is further influenced by destruction of leucocytes so that two factors are at work namely productive and destructive (Ersatz und Verbrauch).

With pyogenic infection there is a bone marrow stimulation to increased production, and also an increased use of neutrophils before they reach normal maturity. The greater the stimulus the less mature are the cells thrown into peripheral circulation. So that, first, the number of the staff forms, then the young forms, and even the myelocytes are found in circulating blood.

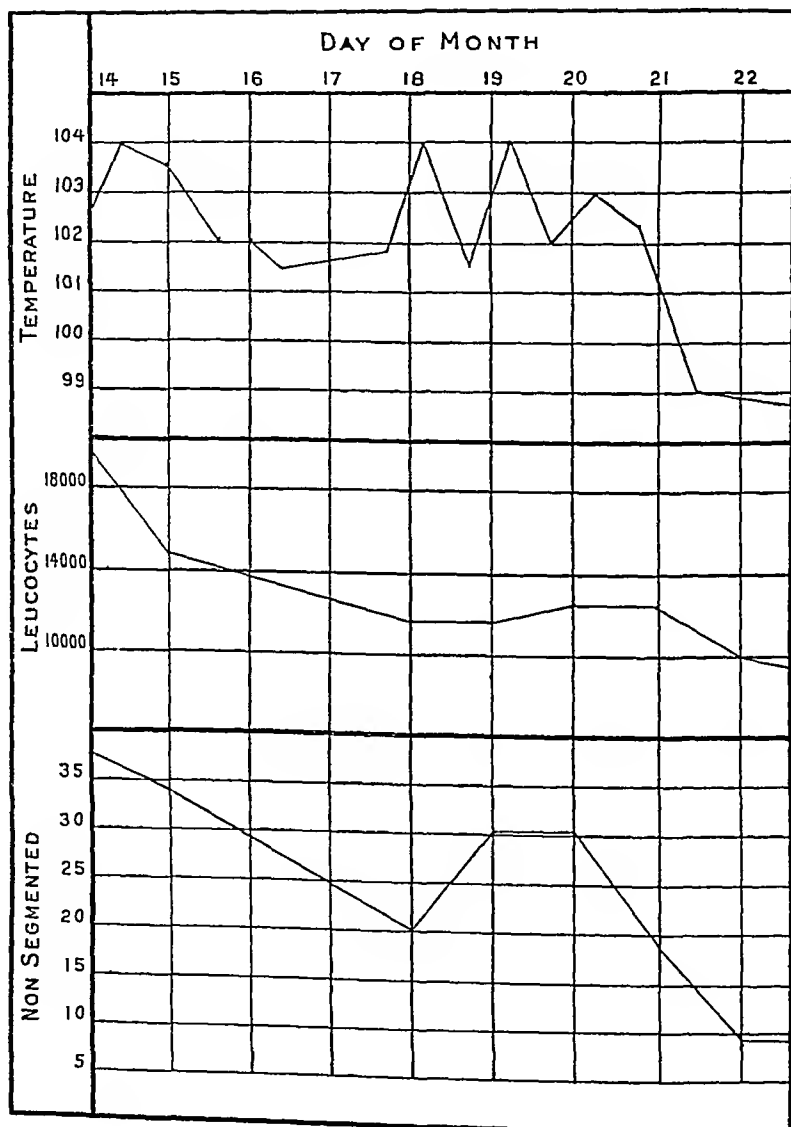


Chart 2

Coincident with this influx of immature forms, the cells are used (Verbiand) before reaching maturity. In classifying these cells according to the nuclear form and consistency, a marked difference in the nuclei is evident. This is designated nuclear deviation (Kernverschiebung). These are usually written from left to right across a page (myelocytes, young forms, staff forms, seg-

mented forms) As the number of cells belonging to the groups on the left is increased, this condition is referred to as deviation to left (Links verschiebung) Changes enumerated above are regenerative changes Besides these some diseases and infections exert an influence primarily inhibiting bone marrow These are degenerative changes

With this conception in mind certain blood pictures indicate certain phases of disease or infection

a Slight stimulations produce only minor changes with slight increase of staff forms

b Moderate stimulus shows a few young forms

c Strong excitants cause marked increase of the young and even parent forms (myelocytes)

Ordinarily the leucocyte count is parallel with the severity of the infection, but with very grave infection a sudden fall may occur In the latter instance the decrease might be interpreted as an omen of recovery but in reality would be the reverse The correct interpretation would be evident by proper scrutiny of the individual cells Undoubtedly many young forms would be present in the blood This phase concerns only the neutrophiles When this ends favorably with a return of nuclear deviation to the right and decrease of leucocytosis there is an increase of monocytes This denotes that the infection has been conquered The healing phase is evidenced by a lymphocytosis Eosinophiles also disappear from the blood in severe infection, and their reappearance is the earliest favorable sign On the other hand basophiles appear only with severe infection when the defensive factors are losing ground and disappear with earliest improvement

Chart 1 shows the temperature and the blood changes in a fatal case of *Staphylococcus septicemia* The increase of the fever the decrease of the leucocytes and the increase of the immature forms foretell a fatal termination

Chart 2 shows temperature and blood changes in a case of pneumonia terminating with recovery It will be noted that while the number of leucocytes drops early the percentage of the immature forms does not return to normal until the fever has subsided

TABLE I  
ACUTE APPENDICITIS

LEUCOCYTE COUNT	AG	EOS	MY	YOUNG	TF	LY	M	NO	TEM	M	K
19,600	0	0	0	0	10	75	15	0			
20,000	0	0	0	0	20	71	9	0			
12,600	0	0	4	0	7	70	19	0			
21,000	0	1	0	0	23	58	18	0			
10,400	0	0	1	2	11	70	10	0			
20,000	0	0	0	0	12	72	15	1			
10,800	0	0	0	0	8	73	19	0			
14,800	0	0	0	0	7	80	12	1			
17,600	0	0	0	2	8	85	5	0	101°		
15,600	0	0	0	1	7	79	13	0			
10,000	0	0	0	4	22	52	18	4	100	Appendix	gangrenous

In all cases of acute appendicitis (Table I), there is a significant increase in the number of the younger cells. Its value was particularly emphasized in the cases which have only a slight increase in the total number of leucocytes.

Only those cases of chronic appendicitis (Table II) are included which showed definite chronic inflammatory changes histologically. These are chiefly characterized by a more or less increase of the lymphocytes.

TABLE II  
CHRONIC APPENDICITIS

LEUCO- CYTE COUNT	BAS	EOS	MYEL	YOUNG	STAFF	SEGM	LYMPH	MONO	TEMP	REMARKS
8,600	0	0	0	0	10	61	29	0		
13,000	0	0	0	2	4	68	25	3		
7,800	0	0	0	0	4	49	47	0		
10,400	0	0	0	0	5	68	27	0		
10,000	0	0	0	0	3	62	35	0		
10,000	1	1	0	0	4	47	45	2		
6,800	0	2	0	2	3	51	42	0		

TABLE III  
ACUTE SALPINGITIS

LEUCO- CYTE COUNT	BAS	EOS	MYEL	YOUNG	STAFF	SEGM	LYMPH	MONO	TEMP	REMARKS
23,400	0	0	0	0	15	69	16	0		
15,000	0	1	0	0	8	58	31	2		
13,800	3	0	0	2	14	57	24	0		
16,000	0	0	0	0	19	55	24	2		
12,000	0	0	0	0	30	50	12	8		
21,300	0	0	0	10	8	77	0	5		

Cases of acute salpingitis (Table III) showed the usual blood picture of pyogenic infection.

TABLE IV  
PREGNANCY

LEUCO- CYTE COUNT	BAS	EOS	MYEL	YOUNG	STAFF	SEGM	LYMPH	MONO	TEMP	REMARKS
9,500	0	0	0	0	10	67	21	2		
14,200	0	1	0	1	3	68	27	0		
18,000	0	1	1	1	7	63	27	0		
9,600	0	0	0	0	8	69	22	1		
11,000	0	4	0	0	12	56	28	0		
9,600	0	0	0	1	8	70	19	1		
14,000	0	0	0	1	4	72	23	0		Toxemia
12,000	0	1	0	0	6	66	27	0		
10,000	0	10	0	0	5	72	12	1		
14,800	0	0	1	0	6	83	0	10		

It will be noted that with normal pregnancy (Table IV) the usual leucocytosis is partially due to an increase of the immature forms.

The changes with acute respiratory infections (Table V) were very irregular. Deviation to left was coincident with the onset of a pyogenic process, usually several days after onset of illness.

TABLE V  
ACUTE RESPIRATORY INFECTIONS

LEUCO- CYTE COUNT	AGE	EOS	LYE	YOUNG	TAFF	S CA	LY P	S N	T A	REMARK
6 000	0	0	0	0	10	32	32	20		Grippe
16 200	0	0	0	0	3	65	28	4		Acute Tonsillitis
11 800	0	4	0	0	2	47	45	2		Bronchitis
11 200	0	1	1	0	1	53	28	0		Pleuritis
9 600	0	0	0	1		38	50	3		Throat Infection
17 200	0	0	0	3	5	70	21	1		Acute Bronchitis
12 800	0	1	5	3	12	20	51	0		Grippe
12 200	0	0	0	0	6	62	24	8		Grippe
11 600	0	3	0	0	17	47	20	8		Acute Sinusitis
8 000	0	0	0	0	4	61	34	1		Acute Sinusitis
8 000	1	0	0	0	10	48	40	1		
15 200	0	0	0	0	4	73	23	0		Acute Sinusitis
10 200	0	1	0	1	3	69	26	0		
14,200	0	0	0	0	5	50	45	0		Acute Sinusitis
38 000	0	0	0	0	21	51	28	0		

TABLE VI  
ACUTE MASTOIDITIS

LEUCO- CYTE COUNT	AGE	VE	Y	LCM	LYN	I	S NO	REMARK
26 000	0	0	3	18	61	1	15	
17 600	0	0	2	8	65	1	20	
15 200	0	0	0	24	50	8	15	
8 200	0	0	0	15	53	28	4	
10 000	0	1	0	20	48	27	4	
11 600	0	0	1	11	61	20	4	
9 800	0	0	0	36	24	26	14	
15 000	0	0	2	11	63	24	0	
12 000	1	0	0	15	50	16	11	
11 200	0	0	0	46	41	10	1	

TABLE VII  
FOCAL INFECTION

LEUCO- CYTE COUNT	AGE	LYE	Y	LYN	M	ON	M	REMARK
8 800	0	2	0	4	64	29	0	Chronic Tonsillitis
7 000	0	1	0	8	74	35	2	Chronic Tonsillitis
7 000	0	1	0	3	64	30	2	Dental Infection
8 200	0	5	0	4	70	32	0	Dental Infection
8 800	0	1	0	7	68	26	0	Dental Infection
11 200	0	0	0	7	62	23	0	Dental Infection
8 800	0	1	0	7	75	41	0	Dental Infection
11 600	0	1	0	4	63	8	10	Dental Infection
7 800	1	0	0	4	62	8		Duodenal Ulcer
11 400	0	0	0	4	67	29	0	Duodenal Ulcer
10 000	0	0	0	17	32	32	15	
10 400	0	0	0	3	50	32	0	Nephritis Acute Focal
8 000	0	0	0	20	68	10	0	
9 800	0	1	0	34	41	18	4	Acute Infectious Nephritis
16 400	1	0	3	14	62	15	3	
15 600	0	0	1	6	68	24	0	Neuritis
13 900	0	0	0	1	6	70	21	Arthritis Acute of Hip
9,600	0	1	0	1	0	44	52	Arthritis Val Heart Disease
6 400	0	2	0	1	2	56	39	Arthritis Chronic
0 600	1	1	0	0	1	51	46	Arthritis Sacroiliac
7 800	0	0	0	1	3	61	34	Mucous Colitis
8 000	0	0	0	1	3	59	31	Mucous Colitis



In acute mastoiditis (Table VI) there was usually a definite deviation. In several cases with low total leucocyte counts the Schilling differential was particularly valuable.

Chronic focal infection (Table VII) is characterized by an increase of lymphocytes.

#### SUMMARY

- A Careful technic is absolutely essential
  - 1 Smears must be thin and even
  - 2 Stains, any good nuclear stain
- B The method is easily learned
 

My experience is that junior medical students rapidly learned to make reliable counts
- C Time to make Schilling differential count is practically the same as for ordinary differentials
- D Results are based on approximately 1000 counts
  - 1 Schilling differential is a sensitive, reliable indicator of the severity of the infection
  - 2 The grade of infection is often indicated when not apparent by the conventional leucocyte count or clinical symptoms

#### REFERENCES

- 1 Cooke and Ponder The Polynuclear Count, 1927
- 2 Schilling, Victor Das Blutbild und seine klinische Verwertung, 1924

#### DISCUSSION

*Dr John W Gray*—How can you differentiate agranulocytic angina from idiopathic aplastic anemias? Is it not possible that the aplastic type of anemia is primary and the throat infection secondary?

*Dr E R Mugrage*—We have done some work with this method at the University of Colorado, and we find very similar results to what Dr Niehaus reports. I would like to ask what technic he uses in counting the cells on the slide. We have been using a method which Dr Bereom brought out last year by counting across the slide at three different points counting 100 cells at each location.

*Dr F W Niehaus* (closing)—In regard to the differentiation between agranulocytic angina and aplastic anemia, it seems that the chief point would be the clinical course. In the former there is a normal red blood picture at the onset. The anemia does not occur until later.

Regarding the method of studying the smear, we use the same method as Dr Mugrage. We pick about four different areas on the slide. Usually one field along the upper, two through the middle, and one along the lower. If I see them bunched up I get a new slide.

# THE SPECIFICITY OF BACTERIA TO THE BACTERIOLYTIC ACTION OF CHEMICALS WITH A NOTE ON THIS APPLICATION TO CHEMOTHERAPY\*

BY ROBERT A. KELTY, M.D., WASHINGTON, D. C.

THE purpose of this paper is to present a report of the apparent specificity or selectivity of antiseptic types of reagents for different bacterial groups with the use of this knowledge for practical chemotherapeutics. Stated the other way around a bacterial type or a flora freshly isolated will undergo bacteriostasis or bacteriolysis when subjected to different substances, all of which have a definite antibacterial coefficient at different times, irrespective of the antiseptic standard of this coefficient for the particular reagent.

For example, the exposure of a stock culture of staphylococcus to a 1:20 solution of carbolic acid will kill in a given period of time. This is taken as the antiseptic standard coefficient of carbolic acid. Exposure of a number of different cultures of staphylococci planted directly from wounds or from culture after isolation on blood agar plates, to a much weaker dilution of carbolic acid, say 1:1000, in a nutrient blood agar for twenty-four hours will show a few cultures growing heavily, some retarded bacteriostasis, and many exhibiting no growth, bacteriolysis. This fact is true for a group of antiseptic substances which I have studied.

Until the World War all work was reported on the standard bacteriolytic coefficient of a given substance as tested out against isolated stock cultures. The memorable work of the Japanese during the Russo-Japanese war, the final development of Dixon's solution after the debate between Sir Almroth Wright and Sir Watson Cheyne during the World War are still fresh in our minds. At that time I reported the antiseptic value of a number of substances worked out after the method of Watson Cheyne. Since the War the recognition of the antiseptic value of some of the dyes, the early successes of mercurochrome and the development of a number of new drugs and chemicals have stimulated work along these lines. One of the ideals of medicine is the development of the ever-increasing group of specific antibacterial substances.

There are a large group of reagents adaptable to clinical therapeutics which show definite bacteriostatic and bacteriolytic action both in vivo and in vitro. In studying these reactions various dilutions of the drugs from 1:1 to 1:500,000 or even 1:1,000,000 may be made and cultures of various types may be subjected to them for varying times. For example a given culture may be inhibited by acriflavine which has a high bacteriolytic coefficient in a dilution of 1:500,000 in twelve hours. Such a series of tests requires a considerable technical setup with many dilutions and cannot be used practically. When a

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Laboratories of the Diagnostic Center U. S. Veterans Bureau, Washington, D. C.

large number of different organisms, maybe of the same type or different types, are used, various dilutions will show varying degrees of bacteriostasis or bacteriolysis in the higher dilutions above the standard bacteriolytic coefficient for that particular substance

While many methods have been advocated and are in use to bring out these points, about two years ago I began the use of a method which gives some reasonable indication of the antibacterial reaction of a number of substances against a single organism or a given flora. The work was stimulated by the increasing use of mercuriochrome, in 1 and 2 per cent solutions, for a wide variety of conditions and with wide variations in results, many of which are most disappointing. The reason for the failures soon became apparent. Too much was being demanded of certain substances, mercuriochrome for example, without a specific knowledge of their limitations and without any variation in their makeup, dilution or strength. Thus mercuriochrome, a perfectly good drug, bids fair to meet the wastebasket of elimination because we are often asking it to do the impossible.

The method used for this study of selectivity or specificity of a drug against a given infection follows. Plain blood agar plates are used for control isolation of the flora under study. A setup of chemotherapeutic tubes is kept in stock. Plain nutrient agar is prepared in 100 c.c. lots in four-ounce bottles for stock. This is melted, 2 per cent whole blood added as for blood agar plates and the antiseptic reagent added in proportion to the final dilution desired for that particular drug. This is thoroughly mixed and the medium poured and slanted into test tubes 100 mm. by 10 mm. For example, when the final tube is to have 1-10,000 dilution of acriviolet, starting with 100 c.c. of stock agar add 2 c.c. of whole blood, 10 c.c. of a standard 1-100 dilution of acriviolet in distilled water. The number of tubes poured will depend upon the amount of work being done. The number of different reagents in different dilutions will depend upon the types and sources of infections with which one is dealing. My work has been mainly on the mouth infections but it may be applied to a number of different sources as will be seen in Table I.

TABLE I  
SPECIFIC CHEMOTHERAPEUSIS

TYPES OF CASES STUDIED	
Gingival Infections	356
Nasal Infections	33
Aural Infections	20
Mastoid Infections	2
Eye Infections, Conjunctiva	11
Abscess	11
Rectal Infection, Sinus	1
Pharynx	13
Blood	2
Abdominal Wound Infections	1
Face Infections	2
Bladder Infections	1
Vaginal Infections	3
Prostatic Infections	4
Total Number of Cases Studied	460
Total Number of Cultures approx	2500

I have studied a large number of substances including the antiseptics commonly used but I have narrowed the final setup to a group of reagents which meet my needs. After testing a large series of dilutions I have taken 1 10,000 as the standard of dilution for twenty four hour incubator reaction with a carbolic acid check of 1 1000 and tricresol check of 1 2000 for my standard of antiseptic coefficient against the organisms under study. If a substance in 1 10,000 dilution shows a constant growth of colonies without even retardation although it may have a constant retardation of growth in 1 100 dilutions it will show an equally disappointing clinical reaction when checked by frequent cultures. On the other hand, the selectivity of substances by retardation of growth against certain flora and the complete inaction in the same dilutions against other flora is a fact which I am unable to explain but which will clinically manifest itself in the same way.

One can have thus prepared on hand a setup of a number of nutrient blood agar slants with different reagents in 1 10,000 dilutions. Material from a case for study is planted directly by loop streak or swab over the surface of each tube in the setup. Care is taken to have approximately the same amount of material planted in each tube. The tubes are incubated for twenty four hours at 37.5 C.

In the early work I planted measured amounts of dilutions accurately. While this is advisable in the careful study of antiseptic values for a drug study, approximate amounts will suffice for a practical comparison. The material for study may be anything pus, mucus, sputum, feces, discharges, skin scrapings, or isolated culture colonies.

Blood agar plates are always planted at the same time for culture control using the same approximate loop quantity. The tubes are read for comparative growth in twenty four hours and the designations of G, growth; RG, retarded growth (bacteriostasis), and NG, no growth (bacteriolysis), used. In a mixed flora one organism may be completely inhibited in its growth while another organism grows profusely. From the standpoint of the case studied this result is charted as growth. The chemical or chemicals in the tube or tubes showing no growth are recommended for clinical use. A number of substances have been tried out and discarded as not practical when they give constant growths in dilutions less than 1 10 000 by this method. Table II shows a group which has been used fairly constantly especially in the gingival work, but which is being changed from time to time to meet conditions. In this table Mer, is mercurochrome, A.F, acriflavine, A.V, acriviolet, G.V, gentian violet, M.B, methylene blue, Agy, argyrol, Met, meta phen, Merpb, mercurophen, Pben, phenol, and Tri, tricresol.

It is not my purpose in this paper to report results except in so far as they illustrate the practicability of the method. Table I shows sources of material with the largest groups from the mouth infections. Table II shows the report of 356 gingival cases, seen during the past year, cases showing evidence of gingivitis with mixed cultural floras in which approximately 2500 cultures have been studied. This table includes the number of cases for each drug used with the number and percentage showing growth, retarded growth and no growth.

TABLE II  
SPECIFIC CHEMOTHERAPEUSIS  
CHART OF 356 GINGIVAL CASES

REAGENT	MER	GV	MB	PHEN	TRI	AGY	MET	AV	AF	MERPH
Total cases	353	354	154	119	34	26	126	355	354	127
Growth	146	168	68	37	8	24	4	8	16	3
Percentage	41	47	44	31	23	92	3	2	5	2
Retarded growth	100	67	37	53	15	2	45	46	66	33
Percentage	28	19	24	44	44	7	35	12	18	25
No growth	107	119	49	29	11	0	77	301	272	91
Percentage	30	33	31	24	32	0	61	84	76	71
Growth and retarded growth	246	235	105	90	23	26	49	54	82	36
Percentage	68	66	68	75	67	100	39	15	23	28

Gingivitis present in one form or another. Mixed bacterial colonies on blood agar plates. Material planted directly from gingival sulci on chemotherapeutic tubes with blood agar control plates. Standard dilution of reagents 1:10,000 with phenol 1:1000 and tricresol 1:2000. Twenty-four hours incubation at 37°C.

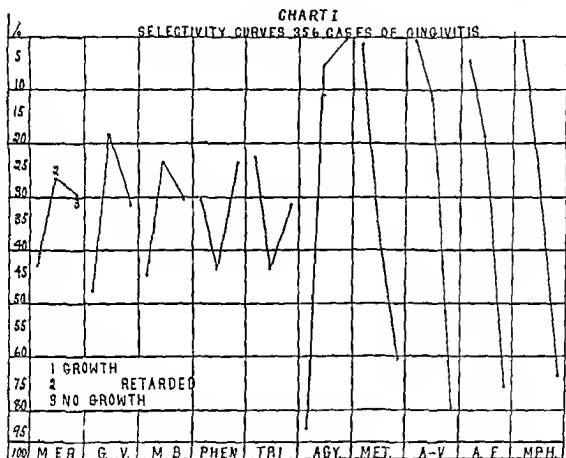
Chart I is a series of curves, showing the comparative standard coefficients of the different reagents against the bacterial flora as it grows from the gingival sulci without reference to the isolated types of bacteria found. The curves show mercurochrome, gentian violet, methylene blue, phenol and tricresol about on a par in the growth, retarded growth and no growth readings, that is a higher percentage of growths than no growth. Aigrol is alone in 92 per cent of growth and never with no growth. Metaphen, acriviolet, acriflavine, and mercurphen are in a group with low percentage of growth and retarded growth and high percentages of no growth.

Chart II shows two curves, A represents the no growths of bacteriolysis, while B represents the growths and retarded growths added together and includes bacteriostasis. The counter position in the curves is made by the grouping of the antiseptic substances.

Chart III is plotted from the six cases taken as illustrations and shows the individual differences in specificity and selectivity of the different reagents. Cases 1, 2, 3, 4, 5 and 6 illustrate the results in individual cases taken at random but with typical variation in readings. Case 1 A-346 Gingivitis. Heavy bacterial flora *Streptococcus viridans*, nonhemolytic streptococcus, hemolytic streptococcus. Growth on gentian violet, retarded growth on mercurochrome, acriflavine, acriviolet, mercurphen and phenol, no growth on metaphen and tricresol. Case 2 A-493 Gingivitis. Light flora small hemolytic streptococcus with scattered staphylococcus. Growth on gentian violet and phenol, retarded growth on mercurochrome, acriflavine, acriviolet, mercurphen, metaphen, and tricresol. No reagent showed any growth. Does not often occur. Case 3 A-504 Gingivitis. Diffuse culture small hemolytic streptococcus with mucoid pneumococcus. Growth on phenol and tricresol, no growth mercurochrome, acriflavine, acriviolet, gentian violet, mercurphen and metaphen. Case 4 A-622 Gingivitis. Moderate cultural flora with a *Streptococcus viridans* and a muddy staphylococcus. Growth on mercurochrome, retarded growth, gentian violet, acriviolet, mercurphen, phenol and tricresol, no growth acriflavine and metaphen. Case 5 A-523 Gingivitis. Pure culture heavy *Streptococcus viridans*. Growth gentian violet and phenol, retarded

growth mercurochrome, acriflavine, metaphen no growth acriviolet, mercurio phen and tricesol Case 6 A 605 Gingivitis advanced Heavy muddy staphylococcus with hemolytic streptococcus, growth gentian violet, retarded growth mercurochrome, acriviolet, mercurio phen, tricesol no growth acriflavine, metaphen and phenol The reason or reasons for this specificity I make no attempt to explain I do not think the type or types of organisms involved as checked against the individual results is the answer but rather the conditions governing the situation

There is a practical application of the method described in this paper which is an attempt at specific bacterial chemotherapeutics When the organisms of a given infection are studied by this method an antiseptic sub

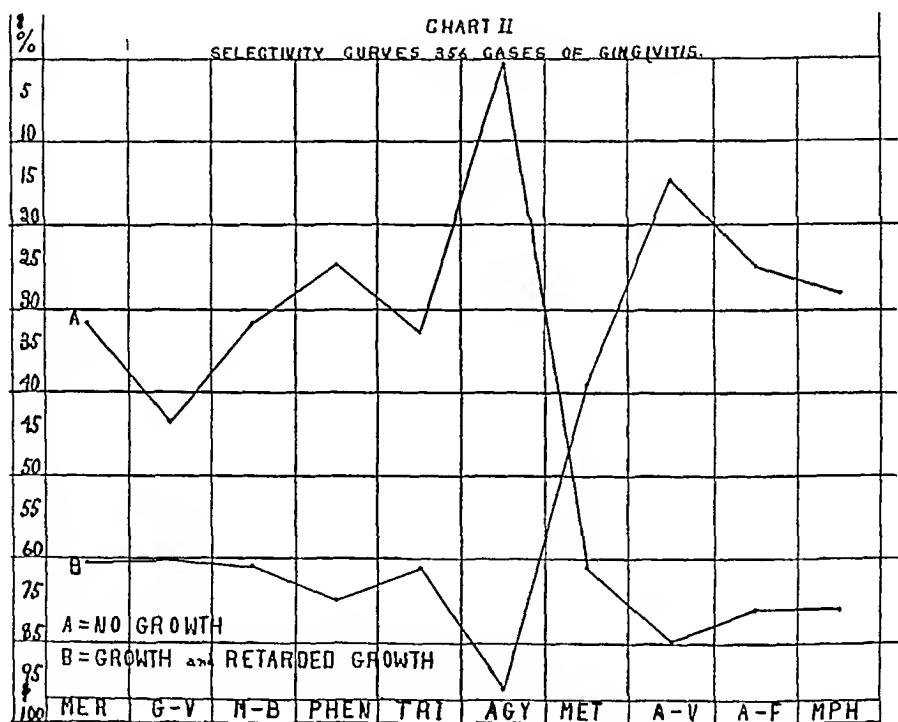


stance, possibly already being used clinically, may be shown to have little or no effect on the retardation of the organisms involved and another substance having an inhibitory reaction may be recommended

In the treatment of infections by the usual routine methods results are obtained in the course of time When these methods are improved by selecting an antiseptic group of reagents having a specific bacteriolytic action on the infectious organisms present and when these substances are applied in dilutions just short of irritation and the applications repeated often enough every hour if necessary over a short period the length of time of repair is not only shortened but the end results are most gratifying The method of application of the drug is just as important as the selection of the drug and the essential feature is that the substance applied must come in constant contact with the bacteria and not be slapped on in a haphazard manner

The dilution of the drug selected is important. It must be used just short of the point of irritation and this must be worked out by experience. For example, acriviolet in the eye will burn slightly in 1-1000 dilution but it can be used and repeated every two hours for a day or so up to its point of irritation when the interval should be increased. In the nose, acriviolet will be tolerated in 1-500 dilution while in the mouth a 1-100 dilution may be applied to the gums and repeated every two hours.

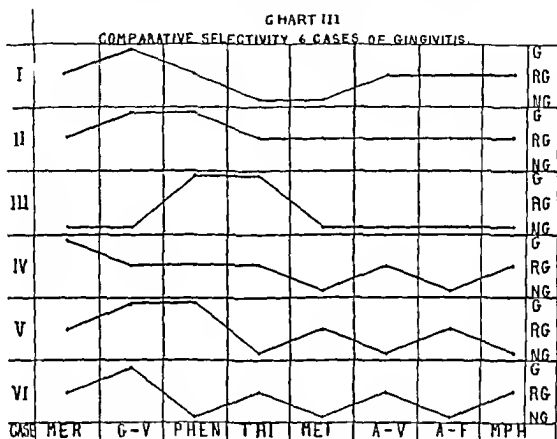
The mercurials, mercuriophen and metaphen, which also have a high standard antiseptic coefficient, may be used in 1-100 dilution in the mouth as a local application for a period of days but used as a mouth wash or gargle in this strength are quite irritating and produce a painful soreness which will



promptly clear up when the drug is stopped. On the other hand, 1-1000 mercuriophen instilled in 5 cc quantities into the urinary bladder may produce such pain that morphine is necessary to control it and yet its antiseptic value is striking. Acriviolet or gentian violet in 1-1000 dilution in the eye may be used while 1-50 may be painted on the skin in erysipelas and repeated every hour without irritating effect.

From these brief remarks and from my experience with various antiseptics over the past two years the following conclusions have been reached. The hit or miss use of any antiseptic, iodine, argyrol, mercurochrome in a straight hundred cases will bring about good results in a high percentage of cases, say seventy-five, will bring indifferent results in about fifteen and will give no results in ten per cent. These results will be surprisingly improved

if the following considerations are worked out for every case. First, the antiseptic substance used must have a reasonably high standard antiseptic coefficient. Second, its selection or specific bacteriolytic action tested out by failure of growth on a nutrient medium with a weak dilution of the drug incorporated in twenty four hours will pick it out as a more or less specific for this particular infection or infections in the given case. Third, the drug must be used in varying dilutions to suit the part of the body involved and these dilutions must be just short of irritation. Fourth, the drug must be applied at intervals frequent enough to control the infections as tested by control cultures, every hour if necessary and not once a day or twice a week. Fifth, the drug must be applied by some means which will assure its coming



in contact with the infectious organisms. I think we have too soon forgot ten the principles that Cattel used with Dakin's solution.

It must be remembered that all so called antiseptic substances have a standard bacteriolytic coefficient as tested out on stock cultures. Such tests involve matters of dilution and require a considerable technical setup. Antiseptic substances are used clinically in a more or less hit or miss fashion, and their selection in a given case depends very much upon their popularity with the clinician at the time. Certain antiseptic substances are used on or in every part of the body and for every conceivable condition of infection and necessarily many of the results are disappointing.

#### CONCLUSIONS

Antiseptic substances may be incorporated in blood agar slants in various dilutions so that direct plants from infected sources may be made and the antibacterial reaction of various substances measured at one time after twenty four hours' incubation at 37.5° C.



These antiseptic substances seem to have a selection or specific reaction to the growth of microorganisms present in a given infection, vice versa, the same organisms present in a series of the same type of infectious condition will react in their growth differently to the same antiseptic in different cases. For this reason a relatively simple method of procedure is reported in this paper whereby this specificity may be determined and a drug selected for clinical usage which will show in culture definite bacteriolysis to the organism or organisms involved.

When this selection is applied clinically up to its point of irritation, when the application reaches the bacteria and when the intervals between application are short enough to stop reactivation of the bacteria, the clinical results are most gratifying.

#### DISCUSSION

*Dr H m Thalhimer*—Dr Keilty's communication is an extremely important one. His method for so easily testing out the susceptibility of organisms to different antiseptics is a definite step in advance.

We have been using the Hygienic Laboratory method for determining the phenol coefficient of disinfectants such as mercurochrome, metaphen, acriflavine, acriviolet, and bichloride of mercury. We found out that the Hygienic Laboratory does not believe that its method should be used in testing out the mercurial antiseptics, but only in testing out disinfectants on a phenol base. In spite of this, for our own interest we thought it would be worth while to find out how some of the commonly used mercurial antiseptics would act under the uniform conditions of the Hygienic Laboratory test. We found that in fifteen minutes a 1:5000 solution of mercurochrome did not kill the typhoid bacilli used. Metaphen killed in dilutions as high as 1:50,000, and bichloride of mercury in dilutions as high as 1:100,000. Under the conditions of this test acriflavine and acriviolet were extremely inefficient. It is only because the results of this study verify in most respects the similar important work of Dr Keilty that they are being mentioned.

*Dr C I Owen*—Have you had any experience with S T 37 solution?

*Dr Robert A Keilty*—This paper deals more with the method than with specific results. In answer to Dr Thalhimer it is very necessary to procure dyes which are as nearly standard as possible, and in having solutions made for clinical usage they should correspond to the lot number used in the laboratory. The antibacterial action or bacterial inhibition varies under the best of conditions and as nearly as possible the same substances should be used in treatment as in the experiments. Reports on S T 37 will appear later.

*Dr F H Hartman*—I would like to ask if the bacteria do not develop a tolerance to the various antiseptics so that bacteriolytic action of a particular chemical changes from day to day?

*Dr Robert A Keilty (closing)*—It is necessary to use freshly prepared solutions. The dyes precipitate out rather quickly and lose their antibacterial action proportionately. Bacteria build up a tolerance to chemotherapeutic reagents and if they are to be used over a period of time it is best to use different drugs at intervals.

# A SYSTEM OF SPUTUM ANALYSIS FOR THE PRESENCE OF ACID FAST BACILLI\*†‡

BY H C SWEANY M D AND ASIA STADNICHENKO A B CHICAGO ILL

THE finding of acid fast bacilli in advanced tuberculosis is one of the simplest laboratory procedures but in childhood, early or latent tuberculosis, it is much more difficult and infinitely more important. In fact a properly performed series of sputum analyses will give more absolute information than any one type of examination.

Heretofore animal inoculation has been used in such cases. Recently reliable culture methods<sup>1</sup> have been devised. In both instances, however, there is a considerable loss of valuable time. It is our purpose therefore to suggest technical improvements in the direct method to re establish it, if possible, as the method of choice in laboratory diagnosis.

If such can be accomplished the advantage both to the physician and the patient is obvious. It will be particularly valuable to Public Health laboratories where large numbers of examinations are made and where the longer procedures are not practicable.

Improvement in the direct method has been made possible by two principles viz a development of concentration of sediment and an apparent increase in bacilli. Other measures are only careful applications of older technical procedures.

Sputum was first concentrated for examination shortly after the discovery of the tubercle bacillus. Biedert<sup>2</sup> as early as 1886 treated a teaspoon of sputum with six to eight drops of sodium hydroxide and examined the sediment. Since then a great many concentration methods have been devised. The various ones may be grouped as the alkali chloride and digestion methods respectively. Only the more important ones will be reviewed here.

Zahn<sup>3</sup> heated sputum with 4 per cent sodium hydroxide and precipitated with normal calcium chloride. Ditthorn and Schultz used 15 per cent potassium hydroxide followed by a liquor ferri oxychlorati. Branner<sup>4</sup> added ammonia, heated on a water bath at 50° C for a few minutes then added ammonium sulphate and a few drops each of alcohol and chloroform, shook and centrifuged. The tubercle bacilli were found in the middle layer above the chloroform. Hammerl<sup>5</sup> used equal parts of sodium hydroxide and ammonium hydroxide then decreased the specific gravity with acetone. Mulhauser<sup>6</sup> used 0.2 per cent sodium hydroxide heated in a porcelain dish and brought near the neutral point with acetic acid without throwing down the mucus. The sedi-

\*This method is an elaboration of the one described by Sweany in the Archives of Pathology (in press).

†Read before the Seventh Annual Convention of the American Society of Clinical Pathologists Minneapolis Minn June 8 9 and 11 1918.

‡From the Research Laboratories of the City of Chicago Municipal Tuberculosis Sanitarium.

of decreasing the number of bacilli shows a greater number than by direct treatment. Many times the actual count is nearly double. Much of the mucus and cellular material is destroyed by incubation (see Tables III to V inclusive).

If a bottle is too full of sputum a cork with an opening in it is exchanged for the first cork and the whole tray is covered with cheesecloth and fastened so that the corks will not blow out. After removal from the incubator the bottles are filled with approximately as much again 3 per cent sodium hydroxide making a final dilution to  $1\frac{1}{2}$  per cent NaOH. The bottles are then placed in the shaking machine and shaken fifteen to twenty minutes, after which they are incubated thirty minutes and finally poured in the centrifuge tubes bearing the same number as the bottles, and centrifugated at about 3000 rpm for eight minutes. The supernatant liquid is then poured off and the drops of liquid allowed to drain completely. The top of the sediment is then scooped off gently with a platinum hoop and smeared thin on a slide bearing the same number as the centrifuge tube. Should the sediment be too small in amount, an albumin fixative may be used to fix it and make the specimen visible. Should there be too much mucoid material still present, as happens occasionally, the sediment is treated with 5 c.c. of 3 per cent hydrochloric acid, stirred and centrifugated again five minutes. This treatment reduces the sediment again over half.

Through the whole process every specimen, tube, and slide is always kept in order. This is a safety check. The slides are placed in a rack and fixed over the electric bath after which they are stained eight to ten minutes by moderate heating, according to Cooper's method<sup>24</sup>. The slides are then washed in tap water in a special tray by means of a rubber tube attached to a water faucet. Decolonization is carried on to completion (ten to fifteen minutes) and the washing is repeated, the counterstain applied and a final washing given. The slides are then dried over the bath and are then examined until they are found positive or for six minutes or more if they are negative. It has been found by experience that in six minutes about 500 to 1,000 fields will have been covered or about one-thirtieth of the average slide. This is the equivalent of two whole slides by the direct smear method because the sputum is concentrated from 50 to 150 times. It is rare that at least one bacillus is not found in the first six minutes if the slide is positive. If one bacillus is found then a search is made until more are found. It is almost a law to state that we do not find one or two tubercle bacilli alone on a slide. It is possible, but with our method of concentration it is almost always possible to find ten or more bacilli. Once in a long time we find as few as four or five. Less than that we make a special point to re-examine until we find more bacilli or declare it only a suspicious result. *One, two, or three bacilli therefore are only considered as a signal for repeated examinations.* Out of thirty thousand examinations, so far, only once has a report of less than four per slide been made, so that it has been necessary to disregard such finding of acid-fast bacilli as error or accident. Many times we have found from four to ten per slide. After a thorough search and repetition of the examination the specimen usually falls automatically into the positive or negative group and the probability of error is small.

After the slides are found positive they are registered in a special record book, are labeled and filed for future reference. The reports are then marked, mailed and delivered directly to the proper place after a duplicate is filed in our records. Within three days from the time the sputum is collected, the reports are received although some may come from thirty miles distant.

To check the various procedures a series of experiments were performed to determine the optimum strength of caustic, to determine whether incuba-

TABLE I  
AVERAGE COUNT OF 20 FIELDS OF INCUBATED AND TREATED SPUTUM

NUMBER	TREATED WITH 3 PER CENT HCl	TREATED WITH 3 PER CENT NaOH
1	0.9	4.0
2	10.0	11.2
3	8.7	21.9
4	4.0	20.2
5	9.3	42.6
6	23.7	100.0+
7	24.1	69.8
8	9.5	100.0+
9	1.0 in many fields	1.0
10	15.6	45.1
11	19.3	48.7
12	12.7	38.1
13	37.2	100.0+
14	1.0 in every two fields	2.0
15	4.1	17.3
16	9.0	50.1
17	5.2	29.4
18	16.2	40.5
19	1.3	4.8
20	9.5	37.2

TABLE II  
AVERAGE COUNT OF 20 FIELDS OF STAINED SMEARS OF SPUTUM INCUBATED IN  
TWENTY FOUR HOURS

NUMBER	CONTROL (UNTREATED)	3 PER CENT HCl FOR 20 MINUTES	3 PER CENT NaOH FOR 20 MINUTES
1	7.2	9.4	21.0
2	5.2	9.0	29.4
3	0.4	11.7	46.0
4	0.5	0.5	25.0
5	2.2	5.3	29.0
6	0.0	12.0	43.9
7	2.4	4.3	19.7
8	2.0	30.1	98.7
9	10.3	--	49.0
10	5.2	--	29.7

tion destroyed any tubercle bacilli, and to test the efficacy of the method in actual practice. Sputums were collected carefully and all work performed in a uniform manner.

Table I shows a comparison of 3 per cent HCl and 3 per cent NaOH as concentrating agents. The NaOH concentrates on the average about five times the HCl.

Table II is practically the same except an untreated control is used for comparison. The HCl concentrates to about half the untreated control. This,

it must be pointed out, is on an incubated specimen that is concentrated from five to ten times by incubation

Table III shows the effect of incubation on sputum treated with sodium bicarbonate and incubated. There is an actual increase in numbers following the incubation. This speaks well for the von Elleimann and Erlandsen method.

Tables IV and V show that the increase on incubation continues to forty-eight hours and that it does not diminish perceptibly in five days.

TABLE III

AVERAGE COUNT OF 20 FIELDS OF STAINED SMEARS OF SPUTUM TREATED WITH  $\text{NaHCO}_3$  AND INCUBATED

NUMBER	3 HOURS	20 HOURS
1	52	103
2	40	70
3	24	37

TABLE IV

AVERAGE COUNT PER FIELD OF 20 FIELDS OF TWENTY FOUR HOUR SPUTUMS INCUBATED TWENTY FOUR AND FORTY EIGHT HOURS RESPECTIVELY

NUMBER	24 HOURS	48 HOURS	NUMBER	24 HOURS	48 HOURS
1	114	179	16	229	322
2	378	440	17	253	300
3	205	239	18	84	107
4	187	230	19	104	115
5	140	270	20	63	87
6	414	464	21	122	197
7	140	151	22	205	278
8	32	72	23	176	224
9	137	205	24	107	171
10	50	38	25	225	84
11	104	85	26	100	251
12	73	82	27	52	73
13	52	60	28	31	54
14	44	27	29	115	198
15	10	30	30	197	386

TABLE V

AVERAGE COUNT OF 10 FIELDS OF STAINED SMEARS OF INCUBATED SPUTUM

NUMBER	24 HOURS	48 HOURS	3 DAYS	4 DAYS	5 DAYS
1	93	125	84	107	171
2	72	100	250	300	253

Table VI shows the amount of concentration before treatment with 3 per cent HCl on twelve consecutive sputums. By treating specimens 125, 127, and 134 with 3 per cent HCl the concentration was increased to nearly fifty times. It can be stated, therefore, that a concentration of fifty times can be assured. We have found by experience that no more than 7 per cent have to be given the HCl treatment in actual practice.

We have also been able to determine the number of bacilli expectorated per day. A dozen slides were carefully prepared, weighed, smeared in the usual fashion, dried and again weighed. In this manner the actual dry weight was obtained. It was found that the average dry smear weighed 0.3

mg each. From this weight, the weight of the actual sputum represented can be calculated by allowing a loss of 90 per cent in weight due to moisture, and by multiplying by fifty the average concentration. This calculation shows that about one to two centigrams of sputum is represented on each slide. Taking the number of bacilli per field and multiplying by the approximate number of fields will give the number per slide or per centigram of sputum. This number multiplied by the centigrams per day will give the total number of bacilli per day. The only variables are the number per field and

TABLE VI  
CONCENTRATIONS OF SPUTUM

NO	QUALITY	QUANTITY	SEDIMENT	PER CENT	TIMES CONCENTRATED
		CG	CG		
123	M I	4.5	0.08	18	36
124	Mucoid	3.5	0.03	08	116
125	Mucoid	2.5	0.13	52	19
126	Purulent	2.0	0.025	12	80
127	Watery	3.0	0.13	40	23
128	Purulent	2.5	0.05	20	50
129	M P	4.0	0.03	0.75	130
130	M P	4.5	0.03	0.70	150
131	Mucoid	3.5	0.13	50	47
132	M P M	3.5	0.03	0.9	116
133	Mucoid	3.0	0.00	20	50
134	M P M	5.5	0.30	10	16

TABLE VII  
SPUTUM RECORD OF TUBERCULOSIS SUSPECTS AT THE MUNICIPAL TUBERCULOSIS  
SANITARIUM DISPENSARY

Patient—J B			Patient—S B		
Result			Result		
1 16 28	negative		4 3 28	+ 15 per slide	
1 25 28	negative		4 4 28	+ 2 per field	
1 27 28	negative				
1 30 28	negative		Patient—P A		
2 4 28	negative		Result		
2 16 28	negative		9 20 27	+ 5 per slide	
2 24 28	negative		9 27 27	+ 1 per field	
3 29 28	negative		Patient—E S		
3 29 28	+ 1 per field		Result		
4 2 28	negative		8 2 27	negative	
4 4 28	+ 1 per field		8 9 27	+ 1 per slide	
4 13 28	+ 1 per field		8 16 27	negative	
4 16 28	negative		12 22 27	+ 1 per field	
4 19 28	+ 4 per field		12 30 27	+ 1 per field	
4 25 28	+ 10 per slide		1 11 28	+ 2 per field	
5 4 28	+ 10 per slide		Patient—P M		
5 17 28	+ 2 per field		Result		
Patient—F B			11 14 27	negative	
Result			3 29 28	+ 11 per slide	
8 4 27	negative		4 12 28	negative	
8 15 27	negative		4 16 28	negative	
8 23 27	negative		5 12 28	negative	
8 29 28	+ 5 per slide		5 25 28	+ 3 per field	
5 17 28	+ 13 per slide		Patient—C H		
5 24 28	+ 16 per slide		Result		
Patient—K G			5 8 28	+ 3 per slide	
Result			5 22 28	+ 1 per field	
1 30 28	+ 11 per slide		5 28 28	+ 1 per field	
5 7 28	more than + 10 per field				

the grams per day For example, if there are two bacilli per field and the patient expectorates 100 gm per day the total number will be 2000 F where F represents the number of fields per slide This number we have calculated at about 20,000 Therefore, the patient would expectorate 40,000,000 bacilli per day By using the Roman M for thousand the patient's coefficient will be 40 MM

Where there are only a few bacilli per slide, the calculation is different If the examiner searched fifteen minutes and found 12 bacilli, he will have covered 3000 fields or  $\frac{3}{20}$  of the slide, as pointed out above There are therefore 80 bacilli per slide or 800 per gm If this patient has 10 gm per day, there will be 8 M bacilli per day No calculations can be made with less than 10 per slide

TABLE VIII

AVERAGE COUNT OF 50 FIELDS OF EMULSION OF TUBERCLE BACILLI TREATED FOR VARYING PERIODS OF TIME

NO	TIME IN HOURS	3% HCl	1% NaOH	2% NaOH	3% NaOH	20% ANTIFORMIN
1	1	68	69	58	52	--
	5	61	60	52	44	--
	5	50	43	37	31	--
2	1	59	55	46	46	--
	1	51	53	45	42	--
	3	39	33	31	31	--
3	5	28	27	20	17	--
	1	165	154	149	151	129
	3	103	101	87	81	--
4	5	95	97	73	70	--
	7	57	55	44	42	--
	15	27	25	24	20	--
5	1	--	73	68	60	48
	2	--	60	56	51	39
6	1	--	79	61	48	--
	3	--	55	49	41	--
7	1	--	77	65	60	--
	3	--	72	60	42	--
8	5	--	52	49	32	--
	1	65	65	--	--	41
9	2	61	58	--	--	25
	3	53	50	--	--	--
10	5	41	43	--	--	--
	1	--	42	38	--	--
11	1	--	38	35	--	--
	3	--	29	30	--	--
12	5	--	24	21	--	--
	1	--	83	89	75	--
13	1	--	80	73	70	51
	2	--	74	69	63	32
14	3	--	67	54	49	--
	5	--	52	47	43	--
15	1	--	77	72	71	--
	3	--	58	51	42	--
16	5	--	50	42	39	--
	24	--	25	17	14	--

In the course of our work we have met with many suspects where only a few bacilli per slide were found or where many negatives were reported for the first few examinations, but they have without exception become frankly positive later

Table VII reveals the reports on seven such patients. It is a remarkable check on the method. One patient has been under observation for nine years but no tubercle bacilli were ever found by the older methods, yet he was found positive on the first examination made by this method.

In order to check the effect of various concentrating agents on tubercle bacilli, a series of experiments were performed where the bacilli were exposed to varying concentrations suspended in 1:10,000 sodium bicarbonate solution for varying periods of time. The results are charted in Table VIII.

In Table VIII it will be seen that the antiformin treatment is much more detrimental than sodium hydroxide and that 3 per cent sodium hydroxide is more detrimental than 3 per cent hydrochloric acid. *But we feel that 1.5 per cent sodium hydroxide destroys very few tubercle bacilli in the time required to complete the test.* This is obviously no comparison of viridity following such treatment.

#### DISCUSSION AND CONCLUSIONS

We are confident that the system reported concentrates sputum as much as possible without appreciable destruction of tubercle bacilli. The method is simple and can be used by anyone in any laboratory. We have constantly borne in mind time saving devices. This has been done to make the test as simple as possible and at the same time rapid because of the large numbers of daily examinations that we have to make.

Objections may be raised against the continued use of glassware because of bacilli that may be carried over. This is not at all valid. By cleaning thoroughly and bathing in chromegemisch, all organic material is destroyed. Then the heating at 220° C. for thirty minutes destroys all acid fast forms. This last step alone is nearly sufficient. We have heated six tubes in which large numbers of bacilli were present and on examination after heating, found no acid fast bacilli.

We feel that Cooper's staining method has gained a permanent place in laboratory diagnosis. We have not found that it gives any false positives if used as the author prescribes and it surely renders the bacilli more distinct.

The question of actually increasing the bacilli in the digestion of sputum is one that cannot be answered positively. It seems from actual count that they increase from two to many fold. This has been done on carefully divided sputum and after a count of many fields. From the appearance of the bacilli it seems that actual division is taking place because they are so frequently found in "nodal" or vegetative forms and in pairs. We must also not overlook the possibility of a sprouting of the granular forms. This does happen in certain specimens according to our own observation. How general it is remains to be proved. It may help to account for the increase in bacilli. Furthermore, the diagnosis of old suspects seems to support this theory.

This method is obviously not intended for sputum with large numbers of bacilli although it is almost as simple. As a method for the examination of suspects however it has no equal at the present time.



## SUMMARY

1 A practical concentration method has been described that concentrates sputum more than fifty times

2 The important feature is autodigestion of the sputum with apparent increase of bacilli

3 It approaches the accuracy of animal inoculation in the early diagnosis of acid-fast bacilli in the sputum

The authors wish to express their gratitude to Misses Barbara Dolezal and Harriet Majewski for their technical assistance

## REFERENCES

- 1 Loewenstein, Ernst Ueber die Reinkulturmethode des Tuberkelbacillus, Wien med Wehnschr 77 767, 1927
- 2 Corper, H J, and Uyei, N Isolation of Tubercle Bacilli From Contaminated Tuberculous Materials, Am Rev Tuberc 16 299 322, 1927
- 3 Biedert, P Ein Verfahren, den Nachweis vereinzelter Tuberkelbacillen zu sichern, nebst Bemerkungen uiber der Farbbarkeit der Bacillen und Aetiologie der Tuberkulose, Berl klin Wehnschr 23 713 717, 1886
- 4 Zahn Ein neues einfaches Anreicherungsverfahren fur Tuberkelbacillen, Munchen med Wehnschr 57 840 842, 1910
- 5 Dittborn, F, and Schultz, W Ein Anreicherungsverfahren fur den Nachweis von Tuberkelbacillen im Sputum, Centralbl f Bakteriol 79 166 172, 1917
- 6 Bruer, Kurt Ein neues Verfahren zur Anreicherung von Tuberkelbazillen im Sputum, Deutsche med Wehnschr 44 266 267, 1918
- 7 Hammerl, Hans Ein Beitrag zur Homogenisierung des Sputums, Munchen med Wehnschr 56 1955 1956, 1909
- 8 Muhlhauser, H Ueber das Biedert'sche Verfahren zum Nachweis von Tuberkelbazillen, Deutsche med Wehnschr 17 282, 1891
- 9 Bezingon, F, Griffon, V, and Philibert Recherche du bacille tuberculeux dans le sang par homogenisation duellat, Compt rend Soc de biol 55 35 37, 1903
- 10 Ellermann, V, and Erlandsen, A Nachweis von Tuberkelbacillen im Sputum, Ztschr f Hyg 61 219 246, 1903
- 11 Matson, R C Der Vergleichungswert einiger neueren Methoden der Sputumuntersuchung auf Tuberkelbazillen des Ziehischen und Muehschen Typus, Beitr z Klin d Tuberk 24 193 215, 1912
- 12 Lange, L, and Nitsche, P Eine neue Methode des Tuberkelbacillennachweises, Deutsche med Wehnschr 35 435 36, 1909
- 13 Uhlenhuth, P, and Kersten, H E Eine neue Methode zum culturellen und mikroskopischen Nachweis von Tuberkelbacillen im Sputum und anderem tuberculosen Material, Ztschr f exper Path u Therap 6 759 776, 1909
- 14 De Lannoise, and Girard Presse Med, Mar 5, 1902 (Cited by Calmette)
- 15 Spengler, Carl Tuberkelbacillenzuchtang aus Bakteriengemischen und Formaldehydesinfektion, Ztschr f Hyg 42 90 114, 1903
- 16 Loeffler, F Ein neues Anreicherungsverfahren zum farberischen Nachweise sparlicher Tuberkelbazillen, Deutsche med Wehnschr 36 1937 1938, 1910
- 17 Bernhardt, Georg Ueber die Verwendung von Antiformin und Ligrone fur den Nachweis der Tuberkelbazillen im Sputum, Deutsche med Wehnschr 35 1428 1429, 1909
- 18 Haserodt, H Neue Methoden zum Nachweis von Tuberkelbacillen im Sputum, Hyg Rundschau 19 699 702, 1909
- 19 Kawai, M Neuere Methoden zum Nachweis von Tuberkelbazillen im Sputum und in pathologischen Sekreten und Geweben, Med Klin., Berlin 7 142, 186, 1911
- 20 Berry, J L, and Smeaton, M A Comparative Tests of Sputum by the Known and Ellermann Erlandsen Methods, J Infect Dis 14 159 161, 1914
- 21 Von Hoesslin, H Das Sputum, 338 343, 1926
- 22 Jochmann, Georg Ueber neuere Nährboden zur Zuechtung des Tuberkuloseerregers, so wie uiber ein neues Anreicherungsverfahren bei der Untersuchung auf Tuberkelbacillen, Hyg Rundschau 10 969 981, 1900
- 23 Hesse, W Ein neues Verfahren zur Zuechtung des Tuberkelbacillus, Ztschr f Hyg 31 502 506, 1899
- 24 Cooper, F B A Modification of the Ziehl Neelsen Staining Method for Tubercle Bacilli, Arch Path & Lab Med 2 382 385, 1926
- 25 Much, H Ueber die granuläre nach Ziehl nicht farbbare Form des Tuberkulosevirus, Beitr z Klin d Tuberk 8 85 99, 1907

- 26 Kioffer, J A New and Easy Method for the Demonstration of Granules in Tubercle Bacilli, *Am Rev Tuberc* 5 662 664 1921
- 27 Herman, M Procédé rapide de coloration du bacille tuberculeux dans les liquides et le tissus organiques, *Ann. de l'Inst Pasteur Par* 3 160 162 1889
- 28 Ziehl, F Zur Färbung des Tuberkelbacillus *Deutschlo med Wehnschr* 8 451, 1882
- 29 Corper H J A Chemical Electrical Fixing and Hot Staining Apparatus, *J A M A* 65 420, 1915
- 30 Sweany H C An Improved Staining and Drying Plate, *J LAB & CLIN MED* 10 929 930 1925

## DISCUSSION

*Dr E D Downing*—We still have to depend upon the tubercle bacilli for the absolute diagnosis of tuberculosis in the chest. With all the improvements in the x ray and in clinical methods, the work reverts to the tubercle bacilli. We must be very careful about finding the tubercle bacilli. We are getting sputum reports as positive in cases of bronchiectasis. There seems to be some increasing source of error somewhere. There seems to be some source of error in the cases they are sending to us about other organisms in the sputum that will remain rod. We teach our technicians that everything red is a tubercle bacillus. We run into diphtheroid organisms in the sputum that are acid fast and we get a report of tuberculosis. It is the same with textbooks giving four or five different methods of staining. It seems that these methods have sources of error in them. This complaint is not so much for doctors as it is for technicians.

For some time we have made routine cultures on Dorset's egg medium. It is surprising that possibly one in ten you will get a growth of tubercle bacilli in three weeks whereas you let the guinea pig run six weeks. I doubt if there are many laboratories that make a routine culture. In smutarium work all the material that comes in to us we run through on Dorset's egg medium. I was glad to hear Dr Sweany speak of the live germs in the sputum. The latest impression is that these germs are dead and that they are not dangerous to other people. I think that is one of the most important things that the tuberculosis worker has to impress on his patient: that these germs they spit up are alive and not dead.

*Dr E E Mudge*—We have a large number of indigent patients in the University of Colorado Clinic. In the past we have also had the large numbers attending the city clinic. While we were taking care of that sputum work we found that the antiformin concentration method was not satisfactory. In fact the late Dr Todd insisted that a proper selection of the particles of sputum to be examined was of more value than the antiformin concentration method and he insisted that we spend the time that we would devote to the concentration method in the proper selection of a caseous mass. At the present time we utilize a paper dish which we coat with paraffin colored with lamp black. With that background we are able to pick out suitable particles with the saving of a great deal of time.

*Dr Robert A Keilty*—It seems to me that these two papers are very timely from the economical point of view. In laboratories with many routine requests for animal inoculations the substitution of cultural methods which will give as many positive results as animal inoculations will mean a financial saving. Just last week I saw a bill for \$127.00 for sixty seven guinea pigs.

*Dr Henry C Sweany* (closing)—In regard to the technicians that do this work. We have two that have been doing it for over six years. I will put them up beside anyone else for speed and accuracy.

I did not mean that we do nothing else but concentration tests. Our routine work is carried on by the routine means as usual. We feel this concentration of the sputum that we have attempted is bringing out more positives than ever before.

## A PHOTO-ELECTRIC HEMOGLOBINOMETER\*

### CLINICAL APPLICATIONS OF THE PRINCIPLES OF PHOTO-ELECTRIC PHOTOMETRY TO THE MEASUREMENT OF HEMOGLOBIN†

BY CHARLES SHEARD, PH D,‡ AND ARTHUR H SANFORD, M D, ROCHESTER, MINN

MANY methods have been devised and used, both in research and clinical practice, for measuring the amount of hemoglobin in the blood. Many critical reviews and comparisons of these various methods have been made, and the advantages and disadvantages, points of accuracy and inaccuracy, and the like, have been pointed out and discussed. It would appear to be of slight if any value, therefore, to rehearse much of what is contained in the literature on this subject.

The method of choice, so far as accuracy is concerned, is the determination of the oxygen capacity of the blood after the manner described by van Slyke. One serious objection to this method is that each determination consumes considerable time, especially if check readings are made. At best, therefore, one person can make, in a day, few determinations of oxygen capacity and hence of the amount of hemoglobin in grams for each 100 cc of blood. The apparatus and method are not likely to be used in a laboratory or institution desirous of making as a routine a large number of determinations on the content of hemoglobin. Neither, on the other hand, is the small laboratory likely to be warranted in maintaining the necessary equipment and technical force.

Spectrophotometric methods are very accurate when employed by those thoroughly familiar with and experienced in such optical procedures. The investigations of Williamson, who used spectrophotometric methods in his work on the effects of age and sex on the content of hemoglobin, have been quoted frequently in the literature and his data on the grams of hemoglobin for each 100 cc of blood have served as an excellent standard and basis of comparison. Davis and Sheard have reported, within the last two years, their investigation on the spectrophotometric determination of hemoglobin, making their readings at wave-length 542 millimicrons using blood diluted 1:200 with a 0.1 per cent solution of sodium carbonate. They report an average variation of 1.3 per cent from the determinations made by the oxygen capacity method of van Slyke. Kennedy also has described recently a method of using selective light filters in connection with a Duboseq colorimeter in measurements on

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‡The author has applied for patent rights covering the use of photo-electric cells in the measurement of hemoglobin and the general principle of the application of such methods with selective spectral filters to the determination of the amounts of unknown substances in solutions. This application for patent rights has been made in order to control the development, accuracy and general serviceability of such devices so that those who acquire apparatus involving the principles disclosed may secure satisfactory equipment. Assignment of any rights granted will be made to The American Society of Clinical Pathologists.

hemoglobin Spectrophotometric methods are very accurate in general and the technical procedures are fairly simple but considerable experience in such procedures is highly essential. All spectrophotometric methods suffer in accuracy from retinal fatigue, condition of adaptation of vision and, in particular, from retinal sensitiveness and the inability to determine absolute equality of the two portions of the field under observation. Some of these points of inaccuracy can be overcome only by introducing other sources of error which may be as great or even greater.

The simpler and commonly used clinical procedures and apparatus have been criticized for one reason or another chiefly, however, because of the variability of standards and the fact that such standards usually do not accurately reproduce color in its three attributes, namely, relative luminosity (brilliance), dominant wave length (hue), and purity (saturation). The Dare method, for example, while it is simple, is not accurate if the hemoglobin content is above 65 per cent and determinations in grams of hemoglobin for each 100 cc of blood cannot be made accurately. It is now almost universally agreed that hemoglobin should be reported in grams for each 100 cc of blood rather than in terms of ambiguous percentages which are based on some arbitrary standard taken as 100 per cent.

All methods in which the hemoglobin is converted into acid hematin either have very changeable standards or if such standards of glass are used, the color is not an exact match for the acid hematin. Osgood and Haskins have devised an excellent acid hematin method, employing a colorimeter for purposes of comparison. Their artificial standard, however, while permanent, varies with temperature so that, at the time of each determination, the temperature of the standard must be taken and a table of corrections consulted in making the final calculations on the content of hemoglobin. Newcomer's method involves the use of a yellow glass filter and while it is one of the most commonly used methods it suffers from the inherent difficulty of color matching.

The ideal method obviously would be one which involves all the advantages and points of accuracy and eliminates the disadvantages and sources of error of other methods. While such an ideal method, in every particular or detail, has not been reached by us in our investigations thus far, we believe that we have devised and are now perfecting an ensemble of apparatus and a method of procedure which approximates in accuracy the oxygen capacity method of van Slyke and which eliminates the inherent difficulties of spectrophotometry, color matchings, variability of standards and the like by the use of a sensitive mechanical eye, the photo electric cell. This cell does not suffer from the sources of error and inaccuracy of the human eye or of different observing eyes. We believe therefore that the application of photo electric photometry to the determination of the content of hemoglobin affords a method which, while it may appear complicated in its theoretical phases can be standardized readily and accurately anywhere and at any time is very rapid, and is accurate (so far as results thus far obtained indicate) to within about 4 per cent (as its maximal error) when compared with the findings by the

wide and 10 mm internal depth or thickness. The only dimension of significance is that of depth, which should be as accurately uniform as possible in a group of absorption cells which would be used in a routine examination of a large number of samples of blood.

*Electrical Circuits*—Fig. 3 contains additional diagrammatic sketches of the electrical connections and adjustments for both the illumination circuit and the photo electric cell circuit. The source of illumination was operated on a six-volt storage cell system, two such storage cells being connected in parallel. It is very essential that the voltage across the lamp which serves as the source of illumination be maintained constant throughout the routine of reading the deflections of the galvanometer included in the photo electric cell circuit.

*Control of Current in the Source of Illumination*—The potentiometric scheme shown in Fig. 3 (A) illustrates how this may be accomplished. A 4.5 volt battery (dry cells) is joined in series with a 1.5 range milliammeter and a resistance of 100,000 ohms. This 4.5 volt battery is so connected as to be opposed to the electromotive force of the main 6 volt battery. By means of the potentiometric device, consisting of 200 ohms (coarse adjustment) and 2 ohms (fine adjustment) in parallel, it is possible to make adjustments such that no flow of current will be indicated on the milliammeter. As long as a null reading of the milliammeter exists (which is determined by an occasional closing of the switch in the circuit containing the ammeter, the 100,000 ohm resistance now being eliminated), the operator is assured of the fact that the voltage across the source of illumination (and therefore the current flowing through the lamp, if the resistance of the lamp filament remains constant) is constant.

*Control of Constancy of Illumination*—This device for the determination of the constancy of voltage across the terminals of the source of illumination however, does not obviate or compensate for any variations which may arise in the circuit containing the photo electric cell and the high voltage battery. Variations in this circuit may occur because of electrical leakage, which may vary with conditions of the weather, slight changes in sensitivity of the galvanometer and the like. This difficulty can be overcome readily by taking some arbitrary value of deflection of the galvanometer in the photo electric cell circuit (such as 400 mm for example, as in our work) when the absorption cell is removed from its position in front of the selective spectral filter in compartment B. If the reading of the galvanometer is either greater or less than the fiducial number, the iris diaphragm (compartment A) is either opened or partially closed and so adjusted as to give a reading on the scale of the galvanometer approximately equal (within a few millimeters) of the arbitrarily chosen standard.

*Galvanometer*—The galvanometer in the photo electric cell circuit is a Leeds and Northrup high sensitivity galvanometer, No. 2285 c, having a resistance of about 550 ohms, a period of about fourteen seconds, an external critical damping of 20,000 ohms and a sensitivity of about  $1 \times 10^{-10}$  ampere for each millimeter of deflection. The protective resistance (Fig. 3, B) amounts to several thousand ohms (10,000 to 20,000 ohms).

the absorption cell and contents to be placed in front of the selective filter or in the optical path of the source of illumination

The photo electric cell, which proved most satisfactory and very constant was obtained from the Research Laboratories of the General Electric Company. This cell has a light sensitive coating of cesium. Such photo sensitive substances when exposed to light, emit electrons. If the photo sensitive film is connected to the negative terminal of the high voltage or B battery (150 to 200 volts) and the inner electrode is connected to the positive terminal and the photo electric cell is then exposed to light the electrons

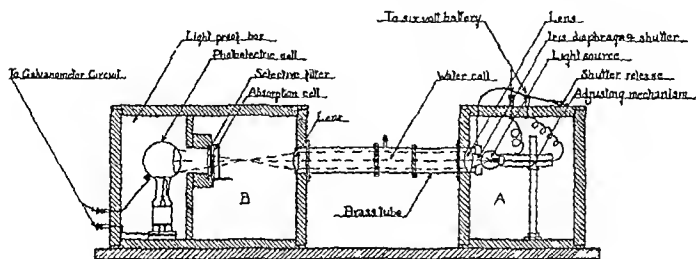


Fig 2.—Diagrammatic sketch (cross section) of the photo electric hemoglobinometer showing the essential features of the ensemble

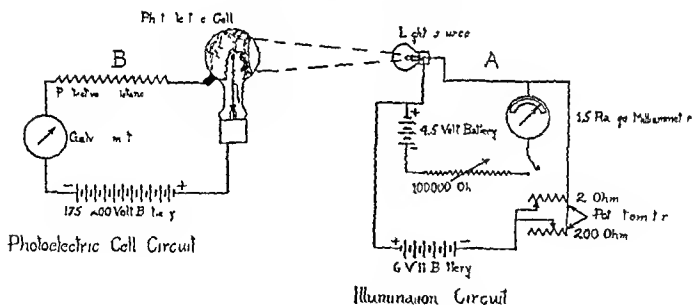


Fig 3.—Diagrammatic sketch showing the electrical connections for keeping the source of illumination constant (A) and the electrical circuit containing the photo electric cell (P)

emitted under the action of the incident light will flow through the cell thereby setting up a current which will be indicated by the deflection of the galvanometer

The selective spectral filter which we have used is known as Laminar No 74 and is the same filter as was used by Sheard and Davis and also by Kennedy in colorimetric methods for the determination of hemoglobin

The absorption cell is of the type commonly used for spectroscopic and spectrophotometric determinations. Its dimensions are 50 mm high, 30 mm

wide and 10 mm internal depth or thickness. The only dimension of significance is that of depth, which should be as accurately uniform as possible in a group of absorption cells which would be used in a routine examination of a large number of samples of blood.

*Electrical Circuits*—Fig. 3 contains additional diagrammatic sketches of the electrical connections and adjustments for both the illumination circuit and the photo electric cell circuit. The source of illumination was operated on a six-volt storage cell system, two such storage cells being connected in parallel. It is very essential that the voltage across the lamp which serves as the source of illumination be maintained constant throughout the routine of reading the deflections of the galvanometer included in the photo electric cell circuit.

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*Control of Constancy of Illumination*—This device for the determination of the constancy of voltage across the terminals of the source of illumination however, does not obviate or compensate for any variations which may arise in the circuit containing the photo electric cell and the high voltage battery. Variations in this circuit may occur because of electrical leakage, which may vary with conditions of the weather, slight changes in sensitivity of the galvanometer and the like. This difficulty can be overcome readily by taking some arbitrary value of deflection of the galvanometer in the photo electric cell circuit (such as 400 mm for example, as in our work) when the absorption cell is removed from its position in front of the selective spectral filter in compartment B. If the reading of the galvanometer is either greater or less than the fiducial number, the iris diaphragm (compartment A) is either opened or partially closed and so adjusted as to give a reading on the scale of the galvanometer approximately equal (within a few millimeters) of the arbitrarily chosen standard.

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## PREPARATION OF THE SAMPLE OF BLOOD

We have tried various dilutions of blood and various types and thicknesses (internal depth) of cell. Experience indicates that the following simple technique is entirely satisfactory. The blood must be laked and the resultant solution of blood (in which the hemoglobin is wholly oxyhemoglobin) must be very clear. Therefore, the proteins must be kept in solution. A 0.1 per cent solution of sodium carbonate is a satisfactory diluent. To cover the range of grams of hemoglobin (extending from conditions of polycythemia to severe anemia) it was found, after many trials, that a dilution of 1:200 was satisfactory in that such a dilution of blood afforded a sufficient range of readings on the photo electric hemoglobinometer when absorption cells of 0.1 cc thickness were used. Such a dilution can be made by adding 0.1 cc of whole blood to 20 cc of 0.1 per cent solution of sodium carbonate. The diluent is carefully measured with a burette and placed in a suitable container. We make use of 50 cc lipped centrifuge tubes for this purpose; such containers make the filling of the absorption cell an easy matter. The pipette in which the blood is drawn obviously must be accurate; we use a serologic pipette, graduated in thousandths of a cubic centimeter, drawing the blood (taken from the vein) exactly to the 0.1 cc mark. All blood is carefully wiped from the outside of the tip of the pipette and the contents blown at once into the solution of sodium carbonate. The pipette is washed out four or five times by drawing up and releasing the diluting fluid. The blood is laked instantly by shaking the tube for a few seconds. The solution of blood is then ready for insertion in the cleansed absorption cell and for obtaining the reading on the scale of the galvanometer in the circuit of the photo electric hemoglobinometer.

## GENERAL PROCEDURE

By way of outlining the routine of procedure which we have adopted we may say that the blood is drawn and diluted in the manner described in the preceding paragraph. The sample of diluted blood is placed in the absorption cell. This cell and contents are then ready for insertion in a suitable containing block so that they rest directly in front of the selective spectral filter. When the necessary adjustments of the illumination circuit have been made and the fiducial reading of the galvanometer secured (with no absorption cell present), the sample of diluted blood is inserted in the instrument and the galvanometer deflection which measures the amount of transmitted light, is read and recorded. From the chart showing the relationship between the grams of hemoglobin, as determined by the van Slyke apparatus and method and the deflections of the galvanometer, the grams of hemoglobin for each 100 cc of blood are read off and recorded.

## THEORY OF THE PHOTO ELECTRIC PHOTOMETER AND SPECTROPHOTOMETRY AS APPLIED TO THE QUANTITATIVE DETERMINATION OF HEMOGLOBIN

In the presentation of the theoretic and practical principles underlying the applications of the photo electric photometer to the quantitative determination of hemoglobin, it is fundamental that the laws of spectrophotometry



be presented and discussed, since both methods afford a means of measuring the transmitted (or unabsorbed) light that passes through any solution under test. A further reason for the presentation of the facts underlying spectrophotometry is the use we have made of this method by way of checks on the determinations made with the van Slyke apparatus and the employment of these spectrophotometric determinations in evaluating the readings of the photo electric hemoglobinometer.

#### SPECTROPHOTOMETRIC DETERMINATIONS OF THE ABSORPTION OF DILUTED BLOOD AND OF THE TRANSMISSION OF THE SPECTRAL FILTER

In Fig 4, Curve 1, is shown the two oxyhemoglobin bands of blood, the alpha band with its center at 578 millimicrons and the beta band with its

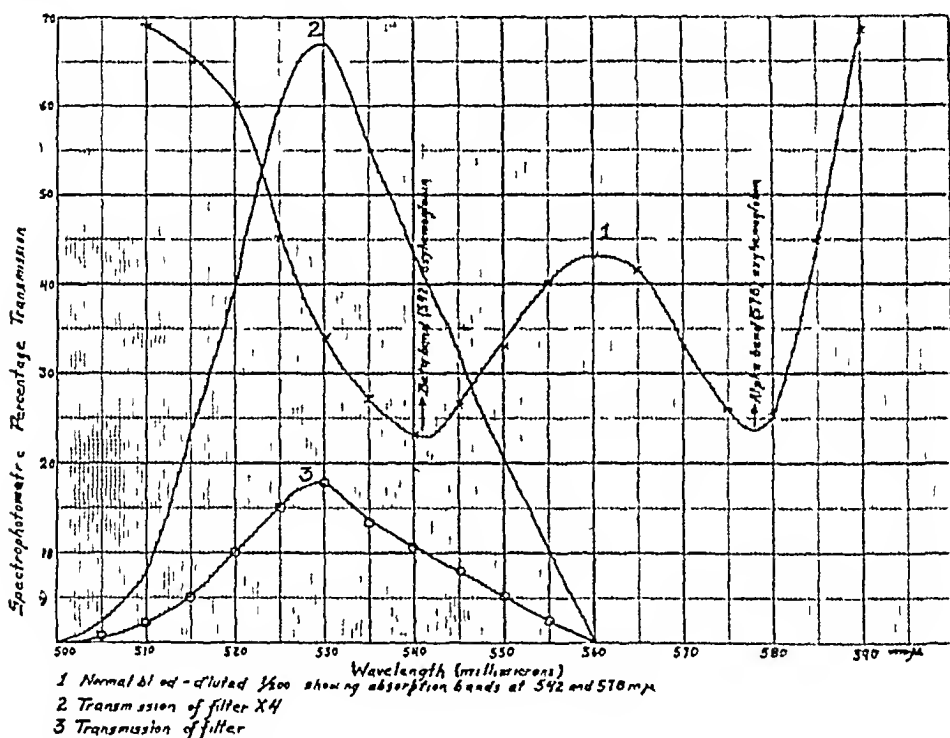


Fig 4—Spectrophotometric data showing the oxyhemoglobin bands (Curve 1) the transmission of the spectral filter (Curve 2) and the transmission of the filter  $\times 1$  (Curve 3)

center at 542 millimicrons. Curve 3 gives the spectrophotometric curve of light transmitted by the spectral filter which could be obtained commercially (and hence easily replaced) and which had the closest approximation to or was the counterpart of the absorption zone of the beta band of oxyhemoglobin. Curve 2 contains the data of Curve 3 multiplied by four. This has been done to show the extent of agreement (or disagreement) of the maximal point of transmission of the filter and the maximal point of absorption as shown in the beta band of oxyhemoglobin. The maximal transmission of the filter lies at 530 millimicrons, that of the beta band at 542 millimicrons.

The band of light transmitted by the filter is not commensurate wholly in shape or maximal point with the beta absorption band and to that extent may be the source of some discrepancy and the cause of part, if not all, of the percentage errors obtained in the use of the hemoglobinometer.

Spectrophotometry has the advantage that all readings on the spectral transmission of various samples of blood can be made monochromatically and at 542 millimicrons for example. This was done by Williamson and again by Davis and Sheard.

The illumination passing through the sample of blood and the light filter and entering the photo electric cell is therefore, not monochromatic in character but is limited to a spectral region determined by the percentages of transmission of light by the blood and by the selective filter when taken together. Such percentages of light transmitted for various wave lengths by the combined solution of blood and the filter can be calculated from the curves of Fig. 4. The spectrophotometric data on a certain sample of diluted (1:200) blood (having approximately 15 gm. of hemoglobin for each 100 cc.) and the corresponding data on the combined transmission of the same sample of blood and the filter as experimentally determined are shown in Table I.

TABLE I

IN COLUMN B ARE SHOWN THE SPECTROPHOTOMETRIC READINGS ON A GIVEN SAMPLE OF BLOOD (DILUTION 1:200) AND IN COLUMN C THE COMBINED TRANSMISSION FOR BLOOD AND FILTER FOR THE WAVE LENGTHS (IN MILLIMICRONS) GIVEN IN COLUMN A.

A	B	C
500	45	20
505		20
510	8	20
518		30
525	32	30
532		0
540	41	38
538		45
535		50
533		50
530	47	50
528		80
525	54	80
523		80
520	59	80
518		75
515	60	50
512		30
510	64	10

These experimental data show that the greater portion of the radiant energy transmitted by the combination of sample of blood and filter lies in the region of 540 to 515 millimicrons, with a maximal value at 530 to 520 millimicrons.

Theoretically, matters might be improved if one could use monochromatic radiation of a wave length approximating the wave length of 542 millimicrons. The green line of mercury fulfills these conditions, but the difficulties incurred in keeping a mercury vapor lamp constant in operation and hence in illuminating power, and at the same time of getting sufficient energy to operate the

photo-electric cell without using a galvanometer of the highest sensitivity (such as a Coblenz) rather rule out this possibility.

The other mode of attack is to develop a filter which will more closely approximate the ideal. This we are endeavoring to do.

We again state that the combined use of spectral filter Eastman 74 and diluted blood gives us the closest practical approach to an illumination of the photo electric photometer with monochromatic radiation and that the photo electric photometer, under these circumstances, summates the energy of the incident light contained in a rather narrow spectral region.

#### THE RELATIONSHIP BETWEEN CONCENTRATIONS OF SOLUTIONS AND THE AMOUNTS OF TRANSMITTED LIGHT

The principles of spectrophotometry as applied to the determination of the amount of a substance in solution can be obtained from textbooks of physical chemistry, physiology, or in the literature in which applications of such principles have been made. Briefly, however, the symbol  $\epsilon$  is used for the coefficient of extinction, so that if  $D_1$  is the depth of solution required to reduce light of a given wave-length to one tenth its value, then the extinction coefficient for this wave-length is

$$\epsilon = \frac{1}{D_1} \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad (1)$$

In practice, it is the intensity ( $I$ ) of the light which is measured. It is more convenient to use a constant thickness of solution and to measure the intensity of the light that has passed through this than to vary the thickness of the absorbing layer. This relationship is expressed in Lambert's law

$$\epsilon = \frac{-\log I}{D} \quad - \quad - \quad - \quad - \quad - \quad - \quad - \quad (2)$$

If  $D$  is kept constant (say  $D = 1$  cm., as in our work), then

$$\epsilon = -\log I \quad - \quad - \quad - \quad - \quad - \quad - \quad (3)$$

From Beer's law, the absorption of light is directly proportional to the concentration, that is,

$$\frac{\epsilon_1}{\epsilon_2} = \frac{C_1}{C_2} \quad - \quad - \quad - \quad - \quad - \quad - \quad (4)$$

It follows, therefore, that

$$\frac{\epsilon_1}{\epsilon_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} \quad - \quad - \quad - \quad (5)$$

Hence, the concentrations (as, for example, relative amounts of hemoglobin) are directly proportional to the negative logarithms of the unabsorbed light.

If, therefore, the grams of hemoglobin per 100 cc. of blood and the spectrophotometric reading for a given solution of blood for a given wave-length of light (say, 542 millmicrons) are known, then it is possible to calculate the grams of hemoglobin of an unknown sample from the combination of the laws of Lambert and Beer given in equation 5. Since successive dilution is equivalent to corresponding reductions in the amount of hemoglobin, it follows that if dilution is plotted against the logarithmic values of the

spectrophotometric readings, a linear relationship should be found to exist. This is shown to be the case in Curve 1 of Fig 5. The percentage of accuracy is all that could be desired.

#### THE RELATIONSHIP BETWEEN CURRENT AND ILLUMINATION IN THE PHOTO ELECTRIC PHOTOMETER

Fig 6 demonstrates the fact that the illumination ( $I$ ) received by the photo electric cell is directly proportional to the current ( $A$ ) set up in the

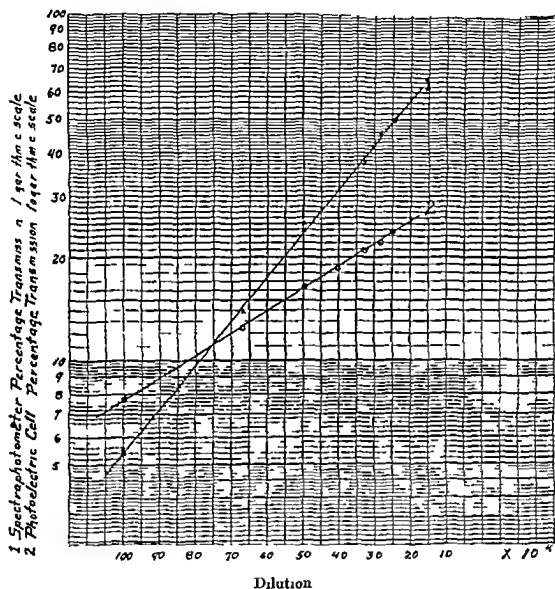


Fig 5.—Curve 1 Showing the linear relationship between dilution of a given sample of blood and the spectrophotometric transmission at 54 $\mu$  millimicrons when plotted on semi logarithmic paper. Curve 2 same relationship when the readings of the photo-electric cell, using the selective spectral filter are plotted against dilution.

circuit containing the photo electric cell and galvanometer provided the voltage remains a constant. Hence,

$$A = KI \quad (6)$$

If the current in the lamp which serves as the source of illumination (Fig 2,  $A$ ) remains constant, then the current in the galvanometer in the photo electric photometer will depend on the amount of light which is transmitted by the combination of sample of blood and spectral filter. Since the amount of light thus transmitted in the spectral region selected is dependent on the concentration ( $C$ ) of hemoglobin in the sample of blood used and

since the laws governing concentrations of material in solutions and amounts of transmitted light indicate that

$$\frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2}$$

it follows that

$$\frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2} \dots (7)$$

This is shown to be the case experimentally in Curve 2 of Fig 5, in which the same dilutions of blood as had previously been examined spectrophotometrically (Fig 5, Curve 1) were tested with the photo electric photometer

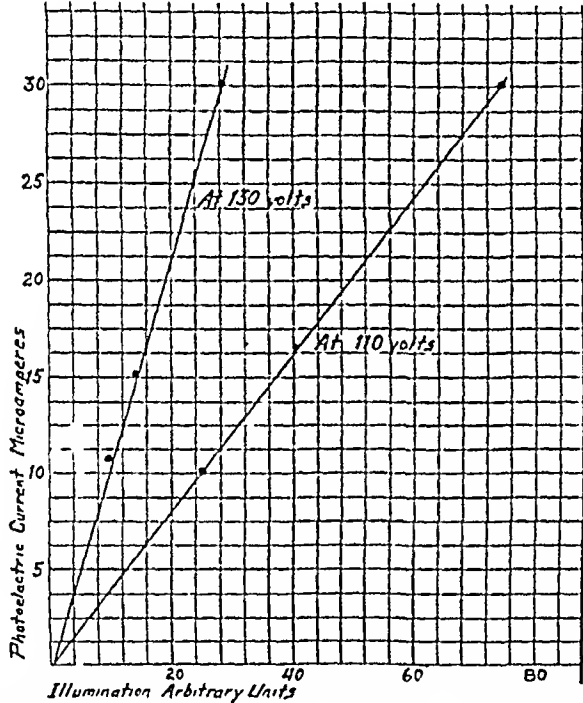


Fig 6—Curves showing that for a given impressed voltage the relationship between Illumination and current (micro-amperes) in the circuit containing the photo electric cell is linear

CORRELATION BETWEEN GRAMS OF HEMOGLOBIN AS DETERMINED BY THE VAN SLAYKE METHOD, THE SPECTROPHOTOMETER AND THE PHOTO ELECTRIC HEMOGLOBINOMETER

The oxygen capacity method of van Slyke gives, by simple calculation and with a probable error of about 1 per cent, the grams of hemoglobin for each 100 cc of blood. Let the van Slyke reading be indicated by V. Then we have the correlation of grams of hemoglobin as determined by the van Slyke method, the spectrophotometer and the photo-electric hemoglobinometer given by the equation

$$\frac{V_1}{V_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2} \dots (8)$$

## EXPERIMENTAL RESULTS

During the course of these investigations we have examined a large number of samples of blood at a constant dilution of 1 200 by the van Slyke, spectrophotometric and photo electric photometric methods. Comparisons of experimental data with the results predicted by theory have formed a considerable portion of our experimentation for the reason that we were cogni-

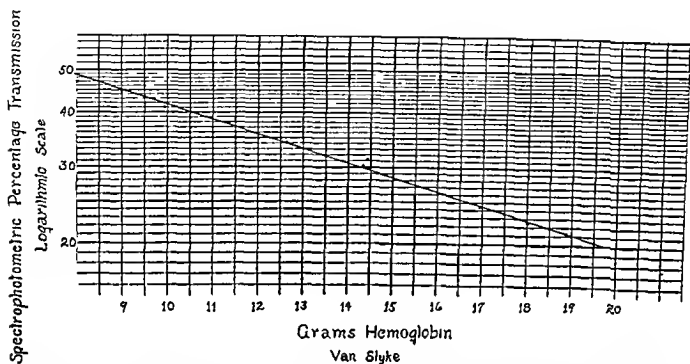


Fig 7—Curve showing that a linear relationship exists between grams hemoglobin determined by the van Slyke method and the spectrophotometric percentage transmissions plotted logarithmically

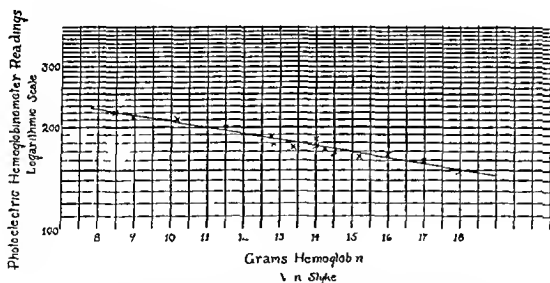


Fig 8—Curve showing the linear relationship between grams hemoglobin and the photo electric hemoglobinometer readings plotted on a logarithmic scale

zant of the possible sources of error in matters of dilution readings on the spectrophotometer, inconstancy and apparent variability of the readings on the photo electric photometer lack of monochromatism of illumination in the photo electric photometer and the like. Radical disagreement between theoretic and practical results led to consistent and careful checking and various attempts at minimizing or eliminating such errors. These attempts to cull out sources of error have resulted in the adoption of the following criteria

1 Dilutions of the blood in the ratio of 1 part blood to 200 parts of diluent (0.5 per cent solution of sodium carbonate)

2 The adoption of an arbitrary or fiducial galvanometric deflection, making such adjustments of the iris diaphragm placed in front of the source of illumination as would give this arbitrarily chosen galvanometric reading when the absorption cell was removed from the instrument and the potentiometric device connected to the light source indicated a null reading on the milliammeter

Figs 7 to 10 inclusive contain data obtained by the three methods used in these investigations. The samples of blood used were selected so as to cover

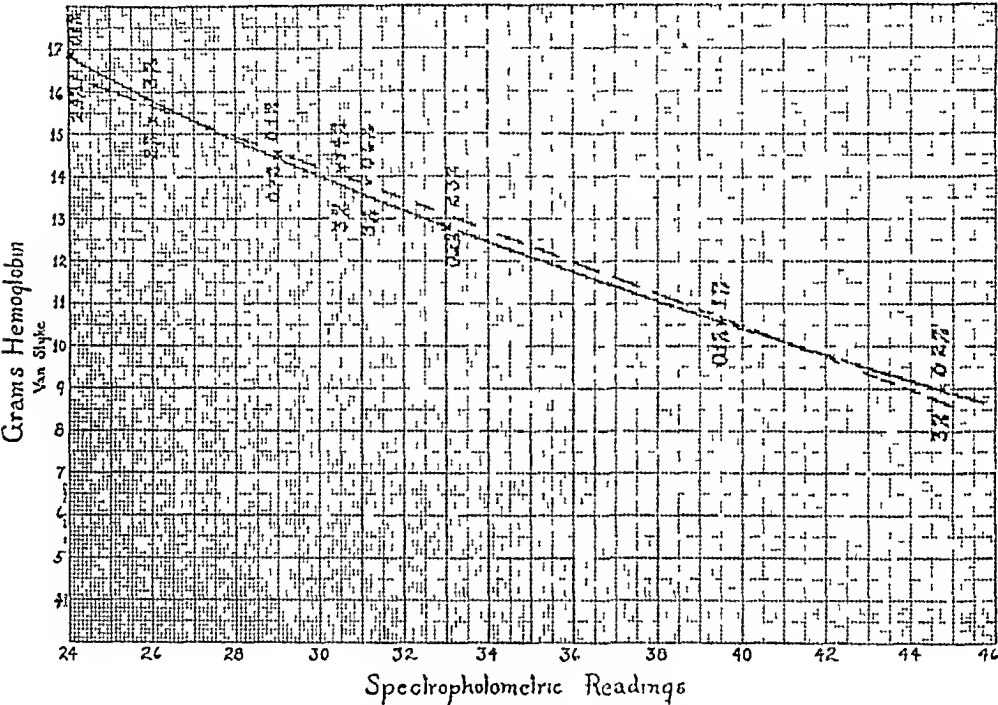


Fig 9—Curves showing the experimentally determined relation between grams of hemoglobin and the spectrophotometric readings for various samples of blood (dilution 1:200)

a reasonably wide range of values of hemoglobin. In the results shown in Figs 7 to 10, preparation and dilution of the blood samples was made by a technician in the laboratories, the readings on the photo-hemoglobinometer were also made by a technical helper in the section of physics. The van Slyke and spectrophotometric readings were made by persons experienced in these fields. Therefore, it is to be presumed that the van Slyke values (which are taken as the standard) and the spectrophotometric readings are as accurate as are likely to be obtained. The routine dilution of blood and the matter of readings on the photo-electric photometer, on the other hand, approximated ordinary working conditions which should improve in accuracy as those who work with them improve in skill. As a matter of fact, however, an inspection of the results obtained by either the spectrophotometer or the photo-electric

hemoglobinometer, when compared with the values on the van Slyke apparatus, shows that the percentage error by either method is not in excess of 4 per cent and averages less than 2 per cent for the group of readings shown (if such an average is justifiable and means anything)

The test of the accuracy of either spectrophotometry or photoelectric photometry as applied to the determination of amounts of hemoglobin lies in equation (8), that is,

$$\frac{V_1}{V} = \frac{C_1}{C} = \frac{-\log I_1}{-\log I} = \frac{-\log A_1}{-\log A}$$

The relationship between concentration (van Slyle) and amount or percent age of light transmitted in a given spectral region by various concentrations

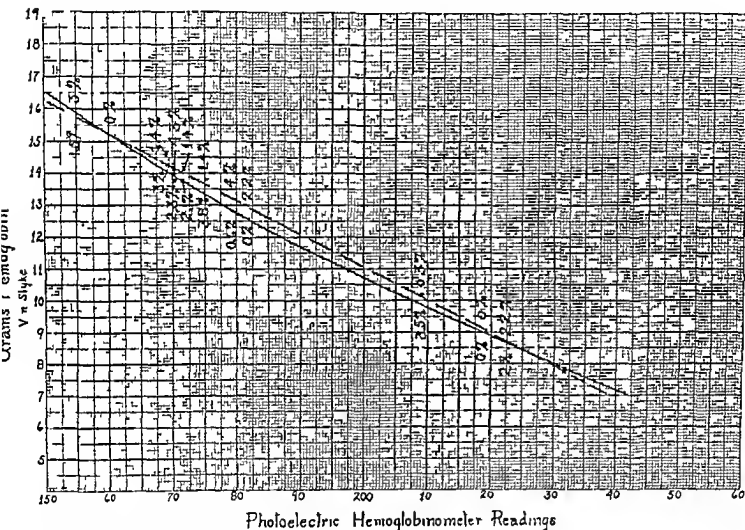


Fig. 10—Curves showing the experimentally determined relation between grams of hemoglobin and the readings of the photo electric hemoglobinometer for various samples of blood (dilution 1/100)

of hemoglobin is such that all points plotted should lie on a straight line when concentrations are plotted against the logarithmic values of the transmitted light. Figs 7 and 8 show how closely theory and practical results agree.

For routine work, however, it would appear to be more advantageous to make charts in which logarithms were eliminated. Figs 9 and 10 therefore, contain the values determined spectrophotometrically and with the photoelectric hemoglobinometer plotted as abscissae and the determinations of grams of hemoglobin by the van Slyke method as ordinates. In drawing the curves, no regard for theoretic considerations has maintained but an attempt



has been made to draw a curve (or curves) which would include the largest number of points. The slightly curved lines in both charts more closely approximate the theoretic form of curve, although it is possible to make determinations of grams hemoglobin within the range of the chart (17 to 8 gm) if straight lines are drawn through the largest number of points possible. To make the final chart and to draw in the nearest theoretically correct and most applicable experimental curve requires the accumulation of a considerable amount of data, particularly covering the additional ranges of polycythemia and severe anemias.

In the case of the curves of Figs 9 and 10, we have indicated the percentages of error, taking the van Slyke values as standards, with reference to both the curved and straight lines drawn in the diagrams. These two charts (Figs 9 and 10) constitute a fair representation of the accuracy of the spectro-photometer and of the photometric hemoglobinometer within a range of 17 to 8 gm of hemoglobin.

TABLE II

COMPARISON OF DATA ON THE DETERMINATION OF HEMOGLOBIN BY VARIOUS METHODS

NO	SEX	AGE	RED BLOOD CELLS, MILLIONS	DAIRY MB	GRAMS, HEMOGLOBIN PER 100 C C			PERCENTAGE ERROR IN PHOTOMETRIC HEMOGLOBINOMETER	
					VAN SLYKE	SPECTROPHOTOMETRIC	PHOTOELECTRIC	VAN SLYKE	SPECTROPHOTOMETRIC
1	M	46	5.02	57	16.91	16.80	16.60	-1.8	-1
2	M	33	1.75	76	15.35	15.60	15.70	+2.3	+0.6
3	F	22	1.80	77	15.21	15.70	15.20	0	-3.1
4	F	31	1.93	78	----	15.30	15.70	----	+2.6
5	F	30	----	----	----	15.10	15.00	----	-0.6
6	F	21	1.16	71	----	11.60	14.20	----	-2.8
7	F	70	4.52	72	14.46	14.50	14.20	-2.0	-2.0
8	F	39	1.92	80	14.30	14.00	14.50	+1.1	+2.6
9	F	51	4.37	69	14.02	14.00	13.10	-4	-4.3
10	F	25	----	80	13.96	13.70	13.90	-0.7	+1.1
11	F	42	3.73	63	----	13.80	13.20	----	-4
12	F	52	4.10	77	----	13.50	11.00	----	-2.6
13	F	51	1.26	71	13.41	----	14.00	+4.5	----
14	F	67	1.15	75	----	14.00	13.70	----	-3.0
15	F	70	3.89	68	12.72	12.70	12.90	+1.5	+1.5
16	F	62	4.28	63	12.68	----	12.70	0	----
17	F	36	4.00	59	10.15	10.10	9.90	-2.5	-1.8
18	F	35	3.86	55	----	10.10	9.70	----	-4.0
19	F	21	3.82	15	9.02	8.80	9.00	0	+2.2
20	F	52	3.17	33	8.56	----	8.50	0	----
21	M	65	3.47	36	----	7.50	7.80	----	+4.0
22	F	57	3.08	39	6.71	10.10	9.90	----	-2.0

Table II contains collected data regarding the sex, age, red blood cell count, Dairé reading, van Slyke, spectrophotometric and photoelectric photometric readings, and percentages of error in a group of routine cases during the two weeks prior to the preparation of this paper.

## SUMMARY

An outline of the data we have presented in this paper is as follows:

1. A brief discussion of various methods of determining the percentage of grams of hemoglobin and their sources of error.

2 A description of a new type of instrument, the photo electric hemoglobinometer, for the accurate and rapid determination of hemoglobin expressed in grams for each 100 c.c. of blood

3 Complete details regarding the *modus operandi* of the photo electric hemoglobinometer

4 The theoretical considerations involved in photo electric photometry as applied to determinations of hemoglobin indicating that the readings on this instrument should follow the laws of Lambert and Beer

5 Experimental data obtained by the van Slyke, spectrophotometer and photo electric hemoglobinometer and their comparison

6 Data substantiating the belief that the photo electric hemoglobinometer will permit of the determination of grams of hemoglobin within less than 5 per cent of the van Slyke determinations which have been used as standards

#### REFERENCES

- 1 DAVIS G E and Sheard Charles The Spectrophotometric Determination of Hemoglobin Arch Int Med 40 226 236 1927
- 2 Exton W G A New Method of Colorimetry J Optic Soc Amer 14 134, 1927
- 3 Haden R L The Normal Hemoglobin Standard J A M A 79 1496 1922
- 4 Haldane, John The Colorimetric Determination of Hemoglobin J Physiol 26 497 504, 1900 1901
- 5 Haldane John and Smith, J L The Mass and Oxygen Capacity of the Blood in Man J Physiol 25 331 343, 1899 1900
- 6 Haskins, H D and Osgood E E Methods of Estimating Hemoglobin Northwest Med 25 500 503 1926
- 7 Kennedy R P The Use of Light Filters in Colorimetry with a Method for the Estimation of Hemoglobin Am J Physiol 78 56 63 1926
- 8 Kera R A Problems of Clinical Hemoglobin Estimation Med Clin N Amer 8 821 831, 1924
- 9 Lindsay J W Rice E C and Selinger M A A Plan for a Standardized Method of Estimating and Reporting Hemoglobin Values J LAB & CLIN MED 11 737 742, 1926
- 10 Newcomer, H S Absorption Spectra of Acid Hematin Oxyhemoglobin and Carbon Monoxide Hemoglobin A New Hemoglobinometer J Biol Chem 37 465 496 1919
- 11 Newcomer H S A New Optical Instrument for the Determination of Hemoglobin J Biol Chem 55 569 574 1923
- 12 Osgood, E E Hemoglobin Color Index Saturation Index and Voluno Index Standards Arch Int Med 37 685 706 1926
- 13 Osgood E E, and Haskins H D A New Permanent Standard for Estimation of Hemoglobin by the Acid Hematin Method J Biol Chem 57 107 110 1923
- 14 Senty E G A Comparative Study of Various Methods of Hemoglobin Determination J LAB & CLIN MED 8 591 604 1922 1923
- 15 van Slyke, D D Gasometric Determination of the Oxygen and Hemoglobin of Blood J Biol Chem 33 127 132 1928
- 16 van Slyke D D, and Stadel, W C The Determination of the Gases of the Blood. J Biol Chem 49 1-42 1921
- 17 Williamson C S Influence of Age and Sex on Hemoglobin Arch Int Med 18 505 528 1916

#### DISCUSSION

Dr Wm G Exton—May I say that I have never presented a hemoglobin method. I have presented a general method of colorimetry which is applicable to many things besides hemoglobin. Its applicability to hemoglobin is demonstrated of course by the fact that Dr Sheard employs my method of colorimetry in his method of determining hemoglobin. Dr Sheard commented on the straightness of the lines in the charts which is somewhat characteristic of the extinction method.

I want to say that I think Dr Sheard's method is particularly beautiful. My extinction method has the defect of varying with different observers, and this defect Dr Sheard has overcome by employing with it what he calls the electric eye. Certainly nothing could be prettier in theory.

*Dr F E Sondern*—Dr Exton has promised us considerable relief and while I hope to live to see this I must say my patience is beginning to wear. I hope we will not have the same experience with this new apparatus. If you can give us some sort of idea as to how much patience we will have to have we would appreciate it.

*Dr Charles Sheard*—The apparatus is still in the experimental stage. The model which we have used in obtaining the results contained in this paper can be improved upon, and we are hoping to do this in the near future. If any patent rights are granted they will be assigned to this Society. The only reason for desiring such patent rights is that we want instruments, when they are placed upon the market, to be as accurate and serviceable as possible. We hope that we may have a satisfactory instrument, suitable for use in any laboratory, ready within the next year.

*Dr Wm G Exton*—If Dr Sheard's experience is at all like mine, I am sorry to have to tell him that when he begins with the apparatus makers he will find his troubles have just begun.

*Dr A H Sanford*—Dr Sondern has had experience with physicists before. Dr Sheard has proved to himself that he is right. I hope that it will be practical to make standard instruments that can be used in institutions.

*Dr Alvin G Foord*—May I ask if anyone has used oxalated venous blood? There is a slight difference in capillary or venous blood in the hemoglobin value.

*Dr A H Sanford*—We tried oxalated blood. The trouble with making dilutions of oxalated blood was that there seemed to be a sedimentation factor that entered in. It would be possible I believe to use oxalated blood if you were willing to take into consideration the slight error due to sedimentation. We used whole blood drawn from the vein and made the dilutions at once using a 0.2 cc pipette graduated in thousandths. Draw the blood to the 0.1 cc mark, wipe off the tip, dilute in 20 cc of sodium carbonate to make a good clear oxyhemoglobin solution free from fibrin precipitate.

## OBITUARIES

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### REPORT OF THE COMMITTEE ON NECROLOGY

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FATE has been very unkind to the American Society of Clinical Pathologists in robbing us, within the past year, of six men who had been valuable members of our organization and whose absence from our ranks will be greatly felt. It is not alone the comparatively large proportion of death loss that we deplore, but the high quality of the men who were snatched from our midst by the Grim Reaper and which gives us greater cause for sorrow.

The mournful news of the death of our Vice President, Lieut Colonel H J Nichols was made more poignant a few days later by the untimely demise of a promising young member, Dr Dean N Beacom of Denver. Another sad demise was that of young Dr Reed Rockwood of Baltimore. In January of this year we lost the beloved author of the most popular textbook on *Clinical Pathology*, Dr James C Todd of Boulder.

The greatest blow to the Society was the sudden death of our valuable secretary and cofounder of the American Society of Clinical Pathologists, Dr Ward Burdick. His spirit still hovers over our meeting and hardly an official action is taken but what our minds revert to the memory of him who by his gentle presence elicited the love and esteem of all his fellow members.

It is also our sad lot to record the passing of Dr Charles E Simon, who through his textbook on *Clinical Pathology* started many of our members on their successful careers in this specialty.

The Committee presents herewith an obituary and some biographical data of each of our departed fellow workers and requests that suitable resolutions of condolence be sent to their respective families.

#### COMMITTEE ON NECROLOGY

PHILIP HILLKOWITZ, *Chairman*

FRANK W HARTMAN

ROBERT A KEILTY

CHARLES C W JUDD

## LIEUTENANT COLONEL HENRY J NICHOLS

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LIEUTENANT COLONEL HENRY J NICHOLS died at Panama September 3, 1927, after an operation for appendicitis. He was born in Milwaukee Wisconsin, May 21, 1877, and appointed in the Medical Corps of the Army from New York, June 20, 1906. He was B A '99 and A M '01 of Yale and graduated in medicine at the University of Pennsylvania in 1904. After passing through the lower grades he became Lieutenant Colonel June 30, 1926 having also held this grade during the World War.

Colonel Nichols was one of the most distinguished scientific men in the Medical Corps. He was Assistant Director of Laboratories of the Army Medi-



LIEUTENANT COLONEL HENRY J NICHOLS

cal School, 1911-1914, and Director, 1923-1926. His name is associated with that of Russell in the development and perfection of typhoid immunization. He was an eminent syphilologist and was a pioneer in the introduction of the use of salvarsan in America. He had been President of the American Society of Tropical Medicine and was Editor of its Journal up to the time of his departure for Panama, where he was, at the time of his death, Medical Inspector of the Department.

He was the author of the work *Carriers in Infectious Diseases*, and of many valuable contributions to periodical medical literature. He was a member of the Phi Beta Kappa fraternity.

Dr. Nichols was a Charter Member of the American Society of Clinical Pathologists, and at the time of his death was Vice-President of this organization.

His loss is greatly mourned by our Fellows.

## DEAN NOLON BEACOM, M D

DR DEAN N BEACOM died suddenly at his home in Denver, September 6, 1927, from a pulmonary hemorrhage. He had been in apparent good health and his sudden demise came as a shock to his many friends and associates.

Dr Beacom was born in Blandsville, Illinois, November 1, 1894. He attended the various public schools in that region, and finally graduated from the Western Illinois State Teachers College.

Shortly after this he came to Colorado for his health. Making his residence at Boulder he attended the University of Colorado, first in the Liberal Arts College from which he received the degree of Bachelor of Arts, and in



DEAN NOLON BEACOM M D

June, 1922, he graduated from the School of Medicine. The following year he spent in the Agnes Memorial Sanitarium. In September, 1923 he became associated with the Department of Clinical Pathology of the University of Colorado, moving to Denver with the opening of the new buildings of the School of Medicine in September 1924, and had continued in this position to the day of his death.

He belonged to the Medical Society of the City and County of Denver, the Colorado State Medical Society, was a Fellow of the American Society of Clinical Pathologists, and was Secretary of the Colorado Society of Clinical Pathologists. He also belonged to the Alpha Tau Omega and Phi Rho Sigma fraternities, and was a Mason.

His death is greatly mourned by our members.

—E R M

## CHARLES EDMUND SIMON, M D

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DR CHARLES EDMUND SIMON was a Charter Member of the American Society of Clinical Pathologists and at the time of his death held the professorial chair of the Department of Filtrable Viruses at the Johns Hopkins School of Hygiene and Public Health

He was born in Baltimore, Maryland, September 23, 1866, and died at his home in Baltimore, November 8, 1927. He came of substantial Teutonic stock. His parents considered that education in this country was inferior to the German system. In consequence his entire precollege education was obtained in Germany. He studied in both Baden-Baden and in Hanover.

The opening of the Johns Hopkins University in his home city attracted him there in his eighteenth year. He took his B A in 1888, majoring in the sciences and particularly enjoying the work of Martin's classes. He began his medical course at the University of Pennsylvania. There he attracted the attention of that keen seeker of talent, Wm Osler. Arrangements were made for the completion of the prescribed medical curriculum at the University of Maryland, where Simon's degree of M D was granted in 1890, while simultaneously he served Dr Osler on his Medical Service at the then newly opened Johns Hopkins Hospital. Here his opportunities were varied. He worked in the neurologic dispensary. He worked on the wards. But most of all he was attracted by the laboratory elucidation of diseased states which he encountered. Osler was quick to appreciate this and acting upon his advice Simon spent a year or more in Paris under Gautier and in Basel under Bunge. It is probable that the hope of an appointment to the teaching staff of the Johns Hopkins Medical School was entertained by Dr Simon, but he returned from abroad with his Swiss bride to assume duties for which provision was not made.

Disappointed in this expectation, he renounced a teaching career and entered private practice. For this he had little stomach, though he soon built up an active following and did much good work as a gastroenterologist. The lure of teaching was strong, however, and he drifted back to Hopkins and worked faithfully there in the laboratories. All this time he was working indefatigably at all odd moments on the production of his text, *Clinical Diagnosis*. The first edition of this appeared in 1896. In the quarter century following, nine subsequent editions made their appearance. Each presented all important new developments in the science of clinical pathology. In clarity of expression, in arrangement of subject matter and explicitness of directions it has few competitors. It is a standard text in every sense of the word.

The ten years following the appearance of the first edition of this work was a decade of discouragement and ill health, during which he led a most sequestered life. The development of fixed fears unfitted him for carrying the burdens of his private practice. His work at Hopkins was abandoned. He was forced to live deprived of all normal human contacts. The summer

days in the open or on a boat on Nova Scotia waters brought back some health, but the weary months of winter again would sap his little store of vigor. The unproductiveness of this period weighed upon him. It was Dr Osler, the student of men as well as medicine who solved the problem. He suggested that an old stable on the property occupied by Dr Simon be converted into a diagnostic laboratory. Note the sapience of that suggestion. The element of hope, the element of occupational therapy, the element of the pioneer were all rolled into one. It was planted in the soul of one naturally imbued with enthusiasm, and the will to do great things. This began what was probably the first private venture of the sort attempted in this country. In this laboratory he and his wife spent much time doing not only the ever increasing volume of work submitted to him by local and distant physicians, but also investigating problems of more purely scientific value. Numerous contributions on many topics emanated from this humble stable. Here, too, he won for himself a country-wide recognition in this field of endeavor. Most of all he began to come out of his state of retirement and seclusion.

The old fever of the teacher fired him. At first it was a chosen few earnest students, who, wishing to perfect themselves in clinical pathology and establish laboratories elsewhere, came to learn first hand from this pioneer. Later he accepted invitations to join the staffs of several local hospitals, organizing their laboratories and stimulating their house staffs to greater endeavor. Finally in about 1910 he burst forth in full swing as a teacher. He became Professor of Clinical Pathology at the College of Physicians and Surgeons, of Baltimore. In 1915 with the merger of this school with the University of Maryland, his medical Alma Mater, he assumed charge of the department in the combined schools. His enthusiasm in the work, his interest in the individual pupil and his assistants, together with his masterly comprehension of his subject made him a teacher of rare quality.

In addition to this teaching, he invariably had some laboratory research under way, the results of which he (alone or often generously including some of his assistants) published in the current literature. Not satisfied with the scope of his first text publication, following the success of Paul Th. Muller's book in Germany, he published the first comprehensive text in this country on *Infection and Immunity* in 1912. This was followed by several other editions. Still further afield, there appeared in 1919 *Human Infection Carriers*. In the same year he epitomized his clear cut and mature ideas of how to present the subject of Clinical Pathology to the undergraduate student in a small *Laboratory Syllabus of Clinical Pathology*.

The last period of Dr Simon's life, though marked by a transition from functional to organic bodily handicap was, none the less, one of deep satisfaction to him and contained more of the joy of living than any previous decade. The Johns Hopkins School of Hygiene and Public Health was started in 1918. Simon soon became intensely interested in its work. His wide clinical knowledge and his training under Martin made him a valuable advisor in the correlation of pure zoology with medicine. In 1920 he began lecturing on medical zoology. Early in the development of the school Dr Welch had planned for a western world publication on hygiene. Simon's experience in



the preparation of texts for publication suggested his fitness for the business management of this enterprise. In 1921 Simon undertook the task of launching the *American Journal of Hygiene* with his unmitigated enthusiasm. Seven volumes of this periodical were published under his discriminating management.

Along with this work he undertook the serious investigation of the rôle of the filtrable viruses in both animal and plant pathology. He collected a practically complete literature on the subject, he lectured and later conducted a laboratory course with the students, he trained new men in this field, he experimented and investigated and he got in touch by letter and personal conference with nearly all investigators on the subject both in this country and in Europe. The development of this twilight zone between chemistry and zoology received such impetus under his guidance, that in 1927 the institution established a separate department and created the first chair of the subject in any university of the United States. Dr. Simon was appointed to this professorial chair.

Imbued as he was with the German idea of scholastic attainment, this appointment in a congenial, stimulating atmosphere of learning was the crown-point of his career. His life of endeavor against odds was at last a success in his own eyes, and there were none more critical. His cup of joy was filled. This appointment was to Simon what the vision of The Child was to Simeon. It was the enumeration of Nine Dninitis.

As a student and a seeker of truth, as investigator of the secrets of science, as writer, editor and above all as a teacher, his death is a universal loss. To those fortunate few who succeeded in knowing him well, his life was an inspiration and incentive to carry on the flame of knowledge and his death is a personal loss.

It is with profound regret that the American Society of Clinical Pathologists records the death of this eminent member.

—C C W J

### PAUL REED ROCKWOOD, M D

DR PAUL REED ROCKWOOD was born in Iowa City, in 1897, and died in December, 1927.

He received his early education at the Morgan Park Military Academy, and his B A and M D degrees from the University of Iowa, the last named in 1922. After serving an internship in the University Hospital he became a Fellow of the Mayo Clinic where he remained for three years. At the time of his death he was Assistant Professor of Medicine in the Medical School of the University of Maryland, at Baltimore.

His early death is deeply deplored.

—P H

## JAMES CAMPBELL TODD, M.D.

JAMES CAMPBELL TODD died at his home in Boulder, Colorado, the evening of January 6, 1928, following a long illness.

Born in Shreve, Ohio, March 17, 1874, he graduated from Wooster College in 1897, with a degree of Bachelor of Philosophy. He continued his studies in the University of Pennsylvania School of Medicine from which he received the degree of M.D. in 1900.

While in Wooster College he held the position of Assistant in Biology during 1895-96. From 1900-01 he was resident physician in the Allegheny



JAMES CAMPBELL TODD, M.D.

General Hospital, Pittsburgh. About this time his health failed and he moved to Colorado, where he located in Denver.

He soon became identified in the field of medical education, first as Assistant in Pathology in the Denver and Gross College of Medicine during 1904-05, then as lecturer from 1905-08, later as Associate Professor from 1908-10 and assumed the professorship of the department in 1910.

On January 1, 1911, the University of Colorado School of Medicine absorbed the Denver and Gross College of Medicine; the two faculties were merged, and Dr. Todd became Professor of Pathology in the Boulder division. He also acted as the secretary of this division until 1916. Since 1923 he has been premedical advisor in the university.

As the study of pathology broadened, he felt that he was losing the con-

tact in the fields of hematology and parasitology he desired. So in 1916 he became Professor and head of the Department of Clinical Pathology which had just been created at his request. These positions he held at the time of his death.

During these years ill health became an increasing handicap, but in the face of these difficulties he showed a determination and persistence of purpose that won the admiration of both students and faculty. His enthusiasm for his work was transmitted to all who had the pleasure to work with him. He was ever willing to aid, and considered the rôle of a teacher as his highest ambition.

Early in his teaching career he found that little or nothing had been compiled in clinical laboratory methods of value to the general practitioner. The collecting and testing of such laboratory procedures became his life work. He kept the viewpoint of the average medical man before him, and simplicity of technique as well as the accuracy of results claimed his closest attention.

His book, *Clinical Diagnosis by Laboratory Methods*, first appeared in 1908, and in the different editions he placed all his writings, with the exception of a few early articles. This book has become established over the world as an authority in its field and it has with few exceptions enjoyed as widespread a distribution as any medical book published in English. He was sole author of the first five editions. The sixth edition, which came out in September, 1927, was written in collaboration with Dr. Arthur Hawley Sanford of the Mayo Clinic. His determination and perseverance may be realized by the fact that the work on all editions of his book was done while bedfast or in a rocking chair.

In recognition of his ability in this field Dr. Todd was the recipient of many honors. Modest to the point of bashfulness, he would seldom speak of his own work except in a reticent manner, but his opinions on diagnostic methods always demanded respect, for they were given only after due consideration, and then in a decisive manner.

He became a Fellow of the American College of Physicians in 1922, was a member of Sigma Xi, and was given honorary membership in Phi Beta Kappa by his Alma Mater in recognition of his ability and learning. He was a Fellow and Charter Member of the American Society of Clinical Pathologists, a Fellow of the American Association for the Advancement of Science, a Fellow of the American Medical Association and an honorary member of the Colorado Society of Clinical Pathologists.

Four years ago he was compelled to give up active teaching, but kept in close touch with the affairs of his department, the university, and the world. He read much, not only in his particular field, but on general subjects. He will be missed in particular by his old students, and by faculty members of the university, who enjoyed his ability as a conversationalist, who admired him for his accomplishments and respected his ability and scholarship. His memory will be cherished as that of a man whom all could well emulate.

It may be interesting to add that Dr. Todd was the first to advocate a Chair of Clinical Pathology in Medical Schools.

—E R M

## WARD THOMAS BURDICK, M D

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THE American Society of Clinical Pathologists has sustained a severe loss in the untimely death of Dr Ward Burdick. In his many years of service to the medical profession in the capacity of clinical pathologist he endeared himself to all who came in contact with him and none could help but admire his gentleness of character and amiability of disposition.

Dr Ward Thomas Burdick was born April 5, 1880, in McKean county, Pennsylvania. He graduated from the National University of Arts and Sciences, in St Louis, Mo. in 1908, and located in Colorado in 1911. Soon after



WARD THOMAS BURDICK M D

his arrival he became interested in clinical pathology and devoted the rest of his life to this branch of medicine. He served successively as laboratory director in the National Jewish Hospital, in the Denver General Hospital, and Children's Hospital. In all these positions, he earned the esteem of his colleagues by his ability, industry, and conscientiousness. He has rendered valuable services by the papers that he contributed on the technic of the Wassermann reaction and other scientific essays.

Dr Burdick's share in the furtherance of scientific medicine of the country is signalized in the formation of the American Society of Clinical Pathologists which he helped to found and of which he was the national secretary from its inception until his death.

Dr Burdick was distinguished by an extraordinarily fine character. He was a stickler for scientific accuracy and precision in technic as is evidenced

by the appearance of his laboratories which were models of orderliness and neatness. His passing will be greatly mourned both by the medical profession at large as well as by his numerous friends in the field of clinical pathology, throughout the United States, all of whom entertain for him a deep sense of esteem and affection.

His endeavors for the success of the organization will always serve as a stimulus and example to our younger colleagues.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D. ABSTRACT EDITOR

## LABORATORY TECHNIC

**BACTERIA** A Practical Method for Single Cell Culture Paine S G J Bact 14 441, 1927

The method is a modification of the old method of separation by attenuation. The attenuations are made in sterile water in a series of five sterile watchglasses. A plate of nutrient agar with the bottom of the dish marked by Chinese ink with nine rows of about 10 spots in each row is placed near by. The suspensions are then examined by placing a loopful of each turn under a cover slip and viewing with the microscope to see that the organisms are not clumped and to form an opinion as to the density of the suspensions. From this inspection, with a little experience one can judge which are the appropriate suspensions to employ. With a steel mapping pen, sterilized by alcohol and flaming the weakest of three selected suspensions is taken and spotted on the surface of the agar to cover three of the marked rows one drop being placed near to but not quite exactly over, each of the ink spots. By just lightly touching the agar the droplet can be made so small that it will easily come within the field of a 2/3 objective and usually one filling of the pen is sufficient to cover the whole of the thirty spots in the three rows.

The plates after inoculation are incubated overnight at a temperature suitable for the organism, and are examined with the low power of the microscope, the dish being placed bottom up, the position of each colony is found by searching the surface of the agar in the neighborhood of the ink spot on the bottom of the dish. If the plating has been satisfactorily carried out one finds that each spot, representing the strongest of the chosen suspensions shows many initial minute colonies say fifteen to twenty, which will later coalesce to form one colony. It seems quite clear under the microscope that most of these initial spots arise from a single cell. Passing down the three rows representing the second of the chosen suspensions one finds the initial spots reduced to a number of one to four with here and there a blank. Then the rows representing the greatest dilution will contain for the most part blanks with here and there a colony with one point of origin or possibly two points but no more. Now it is easy to see that all these colonies which one marks down as having arisen from one point, develop during the next few hours into colonies of precisely the same shape and size. Any in which one detects two points develop into colonies of greater size. One therefore feels that by selecting colonies marked as having one point of origin and neglecting any of these which subsequently develop to more than minimal size one can confidently rely on such colonies having developed from single cells.

**TISSUE** A Simple Method for Staining Reticulum Kinney E M Arch Path & Lab Med 5 No 2, 284, 1928

The solution, which simultaneously fixes the tissue and stains the block for reticulum is prepared by adding 1 gm of sodium sulphantimonate to 100 cc of 4 per cent formaldehyde immediately before using. The tissue should be cut only a few millimeters thick and for the best results should be fresh, although material which has been removed at autopsy several hours after death has been successfully stained by this method.

When this 1 per cent solution is to be used the tissue should be removed after about eighteen hours. The fixing time may be shortened by from nine to twelve hours if a 15 per cent solution is used. In either case, however the tissue should be removed at the end of the stated time, as the stain then seems to begin to disappear. The tissue is then treated as any other material which is to be embedded in paraffin,

with the exception that it cannot be left in the clearing agent more than from one to two hours, whether xylene or cedar oil is used. The stain completely disappears if the material is left in cedar oil for as long as twelve hours. The stain is stable in alcohol, and the tissue may remain in 70 per cent alcohol as long as desired.

After the sections are cut and mounted in the usual manner, the paraffin is removed with xylene and the coverslip immediately attached with neutral balsam.

By this method the reticulum is stained a dark brown and other connective tissue, yellow. An occasional nucleus may be stained. The remainder of the tissue is unaffected by this method and may be stained for routine observation by any of the ordinary stains, such as hematoxylin and eosin. However, hematoxylin or other basic stains obscure the reticulum.

#### RENAL FUNCTION Influence of Posture on Phenolsulphonephthalein Test for Kidney Function, Cordero, N, and Freedman, M. H. Arch Int Med 41 No 2, 279, 1928

The elimination of phenolsulphonephthalein in normal persons is greater in the recumbent posture than during standing. The difference (from 10 to 12 per cent) though definite is not marked, but should be taken into account in making phenolsulphonephthalein tests in borderline cases. The possibility that the difference might be greater in cardiorenal disorders is pointed out.

The effect of recumbency in increasing the volume of output is marked (from 50 to 200 per cent), confirming the results of the earlier investigators.

The probable causes of these postural effects are discussed.

On the basis of fifty-seven experiments on two subjects, at intervals of at least five days, it is shown that the hypodermic route is not as unreliable as is generally believed when the two hour eliminations are taken into account.

The absence of toxic effects during the eight months covered in these studies is offered as one more evidence of the harmlessness of phenolsulphonephthalein. There is no evidence of the development of any increased storage or increased destruction in the body during this frequent and long continued use.

Slight modifications in the technic of renal function tests are suggested, namely, as a result of the foregoing studies and the possibilities mentioned, it seems essential that renal function tests should be made with the patients in recumbent posture to eliminate one more variable, especially in borderline cases. It might be advisable, furthermore, to give water in fractional doses, with a view to assuring at the time of collection of the sample, a volume large enough to stimulate micturition even in nervous subjects, and yet small enough to exclude the possibility of renal inhibition by bladder distension.

#### MILK Use of a Differential Stain in the Direct Enumeration of Bacteria in Pasteurized Milk, Beattie, M. Am J Pub Health 17 1031, 1927

Dead bacteria, stained previously by Loeffler's blue, lose the blue color and become red under the influence of a 10 per cent solution of carbol fuchsin poured rapidly over the preparation. Treated by the same process, the bacteria of a living culture do not give up the blue dye which they have fixed. The variable cyanophilia of bacteria, corresponding to their vitality, appears in a more certain manner if the following mixture is used to stain preparations fixed by moderate heat.

Concentrated fuchsin (Ziehl)	8 cc
Distilled water	100 cc
Loeffler's	100 cc

The coloring mixture ought to remain at least twenty-four hours exposed to the air before being used. It is sufficient to stain during one minute and to wash in water in order for the differential color to appear, blue for the living bacteria and red for those which are dead.

## STAINS Stain Solubilities Holmes W C Stain Technology 2 No 3, 68 1927

The values reported are expressed as grams of anhydrous dye per hundred cubic centimeters of saturated solution Bracketed values are expressed as grams of anhydrous dye per hundred grams of saturated solution

## DYE SOLUBILITIES AT 26 C

	Water	95 Per Cent Alcohol
Martius Yellow (calcium salt) C I No 9	0 05	1 90
Sudan I, C I No 24	Nil	0 37
Sudan II C I No 73	Nil	0 39
Crystal Ponceau C I No 89	0 80	0 06
Orange IV, C I No 143	0 16	0 20
Resorcin Yellow C I No 148	0 57	0 19
Sudan III, C I No 248	Nil	0 15
Sudan IV C I No 255	Nil	0 09
Pararosamin (acetate) C I 676	4 15	13 63
New Fuchsin (chloride) C I No 678	1 1	3 20
New Fuchsin (chloride), Resorcin Addition Product	0 94	5 74
Methyl Violet C I No 680	2 93	(15 21)
Crystal Violet (chloride) C I No 681	1 63	13 87
Crystal Violet (iodide)	0 035	1 78
Crystal Violet (chloride), Resorcin Addition Product	0 28	13 84
Crystal Violet (chloride) Hydroquinone Addition Product	0 50	8 39
Crystal Violet (chloride) 0 Hydroquinone Addition Product	0 79	24 87
Eosin, C I No 768	44 20	2 18
Eosin B C I No 771	39 11	0 75
Erythrosin, C I No 773	11 10	1 87
Methylene Blue (chloride) C I No 922	3 55	1 48
Methylene Blue (iodide)	0 09	0 13
New Methylene Blue N C I No 927	(13 32)	1 65

The various addition products of basic dyes and phenols listed are excellent stains for acid fast organisms and spores which resist staining with basic dyes alone

## B COLI Gentian Violet Lactose Peptone Broth for the Detection of B. Coli in Milk Kessler M A and Swenarton J O J Bact 14 No 1 47, 1927

The medium is as follows

Heat 1 liter of distilled water in a double boiler until water in outer vessel boils Add 50 gm of bile and 10 gm of peptone stirring until all ingredients are dissolved continue boiling for one hour Remove from flame and add 10 gm of powdered lactose Filter through cotton flannel until clear To each liter of filtrate add gentian violet 1 25 000 adjusting the reaction of  $P_H$  7.8 before adding 10 cc of a 1 per cent solution of brilliant green Tube and autoclave for fifteen minutes at 15 pounds pressure

Plain lactose broth, gentian violet lactose broth, and gentian violet lactose peptone bile have been compared as to their reliability for detecting B. coli in milk. It was found that gentian violet lactose peptone bile is the most reliable of these media for this purpose and the formation of gas in this medium when inoculated with milk or diluted milk is a positive indication of the presence of B. coli which for practical purposes needs no confirmation

## TISSUE A New Method For the Rapid Microscopic Diagnosis of Tumors Dudgeon, L. S and Patrick C V Brit J Surg 15 250 October 1927

The tumor or other tissue is cut into and the freshly cut surface scraped firmly with a sharp scalpel The milky juice so obtained is spread evenly on slides and put immediately into Schandinn's fluid while still wet The composition of this fluid is as follows (1) Saturated solution of mercuric chloride in distilled water 2 parts (2) Absolute alcohol, 1 part A few drops of glacial acetic acid are added so as to obtain 4 per cent of the acid in the solution



The wet films are allowed to remain in this fluid from two to ten minutes according to circumstances. If rapidity is essential then an absolute minimum time of two minutes can be employed, but the best preparations are obtained by ten minutes' fixation. In some cases the wet films were placed in warm Schaudinn's fluid, but no advantage was obtained. Time will be saved if the tissue is scraped and the wet films are put into the fixative in the operating theater, so that fixation will take place during transit from the operating theater to the laboratory. The films are afterwards washed in spirit, and then in distilled water. Mayer's hemalum is employed as the nuclear stain, and eosin as the counterstain. Dehydration and clearing are done with absolute alcohol and xylol, and the films are then coverslipped with Canada balsam. The total time required for the technical process can be as short as eight to ten minutes, but the time required for the microscopic examination is dependent upon the nature of the tissue and the rapidity of the investigator.

**BACTERIOPHAGE** A Method of Isolation, De Groot, A. F. J. Immunol 14 No 3, 175, 1927

The organisms from 36 cc of a twenty-four hour B. coli culture were concentrated to 4 cc. To this were added 5 cc of a B. colibacteriophage filtrate and after shaking carefully the mixture was placed in the refrigerator three hours. The tube was then transferred to a bath at 60° C for fifteen minutes, cooled, and centrifuged at high speed for twenty minutes. The supernatant fluid was discarded and the organisms were washed three times with cold, normal salt solution and suspended in 10 cc of distilled water. The latter operations were carried out as rapidly as possible. After twenty-four hours in the incubator at 37° C the suspension was filtered through a fresh Berkefeld bougie and the filtrate preserved as "pure bacteriophage." Titrating this solution it was found to cause lysis in a dilution of  $5 \times 10^6$ . The original filtrate produced lysis in a dilution of  $9 \times 10^6$ .

Carrying this method out on a larger scale and washing the organisms only once with a large volume of saline, 50 cc quantities of "pure bacteriophage" solutions have been prepared which gave lysis in a dilution as high as  $11 \times 10^6$ , original filtrate,  $10 \times 10^6$ . This was done with the ordinary centrifuge of 300 cc capacity.

**POLLEN EXTRACTS** The Preparation and Purification of Pollen Extracts, Alles, G. A., and Lawson, R. W. J. Immunol 14 No 3, 181, 1927

Two grams of desiccated pollen is extracted, with occasional shaking for twenty-four hours or longer, with 100 cc of the following glycerol extracting fluid mix:

1 volume 0.30 M sodium dihydrogen phosphate (11.4 gm  $\text{NaH}_2\text{PO}_4$ , 12  $\text{H}_2\text{O}$  per liter)

4 volumes 0.30 M disodium hydrogen phosphate (107.5 gm  $\text{Na}_2\text{HPO}_4$ , 12  $\text{H}_2\text{O}$  per liter)

5 volumes c p glycerol

The pollen extract is filtered through a Berkefeld filter. It is 1 to 50 with respect to extracted pollen and contains 20,000 pollen units per cubic centimeter. (The unit being defined as the active pollen substance extractable from 10<sup>6</sup> gm pollen, first defined by Noon for timothy pollen.) The extract contains 50 volumes per cent of glycerol, is isotonic in salt content, has an hydrogen ion concentration within the limits for normal blood sera ( $\text{pH}$  7.4) and has the maximum buffering effect for this  $\text{pH}$  that is obtainable with an isotonic salt content. Extracts made with this solvent and the similar one using mono and dipotassium phosphates have retained satisfactory clinical activity for a period of two years and within the limits of error of skin testing methods appear to contain all of the salt and water soluble active substance present in pollen gran. The concentration of glycerol present is somewhat irritating on injection but not sufficiently so to be harmful. It is believed that glycerol is useful to insure stability, and practical sterility is secured by this concentration.

**COLLOIDAL GOLD SOLUTION** A New Method of Preparation Wuth O and Faupel  
M Bull Johns Hopkins Hosp 40 No 5, 297, 1927  
The mode of procedure is as follows

**REAGENTS**

- 1 per cent gold chloride (Merek)
- 3 per cent potassium carbonate
- 25 per cent formaldehyde (prepared from Merek's 37 per cent formaldehyde 675 cc made up to 100 cc)
- 01 per cent tannic acid
- 04 per cent phenol red (alcoholic)

Chemicals should be pure and glassware perfectly clean

Preliminary titration To 100 cc distilled water add 1 cc of gold chloride solution and 2 drops of phenol red indicator then add potassium carbonate solution until a definite pink remains from one to one and one half minutes (usually 05 cc)

Preparation of Colloidal Gold Place 250 cc distilled water over bunsen flame add 25 cc gold chloride and an amount of potassium carbonate calculated in the previous titration after shaking add 1 drop of 01 per cent tannic acid Heat until solution boils vigorously Remove flame Add 15 cc formaldehyde quickly and shake continuously until solution turns a clear deep red The temperature should be kept close to boiling point until the final color change has taken place When the solution has cooled to room temperature it should be tested with a known parietic and with a normal spinal fluid

**TISSUE TECHNIC** A Rapid Method for Zenker Fixed Tissue Hoag F H and Jacobson V C Arch Path & Lab Med 4 No 3 397 1927

For fixation a piece 2 by 1 by 0.3 is placed in 80 to 100 cc of fixing fluid

The following steps are then observed

- 1 Fix in Zenker's fluid for three hours
- 2 Wash three hours (Usually removes all bichromate but no harm is done if a trace remains as it will be removed by washings of the section)
- 3 Place in 80 per cent alcohol for one half hour
- 4 Place in 95 per cent alcohol for one half hour
- 5 Place in absolute alcohol one half hour
- 6 Place in second absolute alcohol one half hour
- 7 Chloroform one half hour
- 8 Saturated solution of paraffin in chloroform one half hour
- 9 Place in oven for ten minutes
- 10 Embed at 53 C for from twenty to thirty minutes

Cutting and staining is done as follows

- 1 Cut sections and mount on slide by albumin glycerin method
- 2 Place slide in an upright position in oven for twenty minutes
- 3 Draw slide over edge of a hot plate several times to assure secure mounting
- 4 Chill by holding slide on ice and blot (Steps 3 and 4 occupy five minutes)
- 5 Place in xylol for five minutes
- 6 Dip into absolute alcohol Dip into 95 per cent alcohol Dip into 80 per cent alcohol
- 7 Place in 05 per cent iodine for five minutes
- 8 Decolorize in four changes of 80 per cent alcohol
- 9 Delafield's hematoxylin from ten to fifteen minutes
- 10 Acid alcohol to remove any excess of hematoxylin Wash in water (Usually there is no excess of hematoxylin and this step is unnecessary)
- 11 Restore hematoxylin blue with weak ammonium water Wash in water
- 12 Place in eosin or phloxine then in absolute alcohol then in xylol
- 13 Mount in xylol balsam

## REVIEWS

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Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,  
Richmond, Va

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### *Diseases of the Skin\**

A TEXTBOOK and reference manual on diseases of the skin written by a man eminent in his profession, and worthy of bearing his name. The illustrations are abundant and excellent. The text follows the general plan of the preceding edition. It is stimulating to find a textbook on dermatology in which the term eczema has been eliminated. "The term eczema means absolutely nothing. Practically all cases of eczema in the adult are examples of dermatophytosis of anaphylactic dermatitis, of dermatitis venenaria."

In the preface the author announces that the subject of anaphylactic dermatitis has received considerable mention. When we turn to the text however we find what the reviewer considers an altogether too brief discussion. We are still waiting for the textbook on dermatology which will present an adequate discussion of the relation of anaphylaxis or allergy to cutaneous manifestations.

### *Physical Diagnosis†*

A COMPREHENSIVE textbook of diseases of the lungs, circulatory apparatus, and abdominal organs with brief additional chapters on regional diagnosis in the head, neck and extremities and on examination of the nervous system. This work is now in its fifth edition.

### *The Pneumothorax and Surgical Treatment of Pulmonary Tuberculosis‡*

THIS volume is primarily a work on pneumothorax. In it are presented the history, mode of action, indications, counter indications, and technique of artificial pneumothorax and similar procedures together with a short chapter on thoroplasty and other surgical measures.

### *Bacterial Vaccines and Their Position in Therapeutics§*

RATHER a difficult subject to cover in eighty three pages especially when one tries to mention all conditions in which vaccine therapy has been tried. The book gives a good bird's eye view but at the end leaves you like the bird, up in the air. However, this weakness

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\**Diseases of the Skin* By Henry H. Hazen A.M. M.D. Professor of Dermatology in the Medical Department of Georgetown University. Professor of Dermatology in the Medical Department of Howard University. Sometime Assistant in Dermatology in the Johns Hopkins University. Member of the American Dermatological Association. Third Edition. Two hundred forty eight illustrations including two color plates. Cloth. Pp 571. The C. V. Mosby Company St. Louis Mo. 1927.

†*Physical Diagnosis* By W. D. Rose M.D. Associate Professor of Medicine in the University of Arkansas. Little Rock Ark. Fifth Edition. Three Hundred Ten Illustrations and Three Color Plates. Cloth. Pp 819. The C. V. Mosby Company St. Louis Mo. 1927.

‡*The Pneumothorax and Surgical Treatment of Pulmonary Tuberculosis* By Clive Riviere M.D. Lond. F.R.C.P. Physician City of London Hospital for Diseases of the Heart and Lungs. Victoria Park E. Physician E. London Hospital for Children. Shadwell E. Second Edition. Illustrated. Cloth. Pp 311. Oxford University Press. American Branch New York. 1927.

§*Bacterial Vaccines and Their Position in Therapeutics* By Leonard S. Dudgeon C.M.G. C.B.D. F.R.C.P. Lond. Professor of Pathology. University of London and Director of Pathology St. Thomas Hospital. Hon. Colonel A.M.S. Cloth. Pp 57. Price \$2.50. Paul B. Hoeber N.Y. 1928.

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NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

is inherent in the problem rather than in the author for six hundred or a thousand pages of utmost detail at the present time would in the end leave its reader in very much the same position. Of course, this does not apply to subjects such as typhoid or smallpox vaccination.

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### *International Clinics for December 1927 and March 1928\**

WE CONGRATULATE the editors and publishers of *International Clinics* upon the completion of thirty seven years of uninterrupted publication during which one hundred and fifty volumes have been issued.

The last volume for 1927 contains a collection of twenty five selected clinical lectures delivered before the Interstate Post Graduate Medical Association of North America during its European tour.

Both of these volumes follow the established procedure with a wide diversity of subjects and interests with sections devoted to diagnosis and treatment, medicine, pediatrics, surgery, pathology, the medical specialties, medical history and a review of progress in medicine during 1927.

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### *Diseases of the Mouth †*

THE first one hundred and eighty seven pages are devoted to maladies of the teeth and gums, the presentation being made with a view both to the local pathology and the remote systemic effects.

The remainder or greater portion of the book discusses most exhaustively diseases of the lips, the tongue, the throat, the salivary glands, the maxillary sinus, the palate, the jaw bone, and the mouth as a whole (stomatitis). There is a chapter on specific infectious diseases, infections of the floor of the mouth and of the neck, one on diseases of the nerves, another on diseases of the blood and blood vessels in their relationship to local manifestations in the mouth, and additional chapters on fractures, tumors, cysts, and the relationship of oral sepsis to systemic disturbances. The volume is remarkably comprehensive and should serve as an excellent reference manual.

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*International Clinics*, Volume IV, Thirty Seventh Series, 1927. Pp. 309. *International Clinics*, Volume I, Thirty Eighth Series, 1928. Illustrated. Cloth. Pp. 307. J. B. Lippincott Company, Philadelphia, Pa.

†*Diseases of the Mouth*. By Sterling A. Mead, D.D.S., Professor of Oral Surgery and Diseases of the Mouth, Georgetown Dental School, with 4 original illustrations in the text and 31 full page color plates. Cloth. Pp. 35. The C. V. Mosby Company, St. Louis, Mo. 1928.

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Editor WARREN T VAUGHAN, M D

Richmond, Va

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## EDITORIALS

### Clinical Laboratory "Bunk"

IT IS a sad fact that as time has gone on and as clinical laboratory methods have been perfected, clinicians have seen in the improvements, opportunities for saving time by means of technicians. Many of them, the clinicians, not the technicians, think that in "the run of the mine" the laboratory will make a diagnosis in less time than they can, and so they make requests for "routine" or for "complete" tests. In not infrequent cases the clinician has a series of tests which he calls "routine" which may mean anything and which may include not only the usual qualitative urine and blood tests but also a whole series of quantitative studies embracing special blood counts and blood chemical estimations including the ureters index, cholesterol, calcium and carbon dioxide combining power. In some offices and in some institutions the laboratory studies precede the physical examination!

When such a situation exists there is good reason for *debunking*, to use

an ultramodern verb for as every good clinical pathologist knows, most of the blood chemical laboratory work called for is *bunk*, to use the expressive noun

The situation is that "the average physician in general practice will not see more than ten or a dozen cases a year in which a chemical analysis of the blood will be of any value to him in diagnosis or treatment."

Nowhere in recent literature has the state of affairs in respect to blood chemistry been exposed with greater clarity than in the article of Reed Rockwood on Chemical Tests of the Blood

The commonest mistake says Rockwood is to order a "routine blood chemistry," a term which has no meaning. Such a term really means that the clinician does not know anything of the indications for chemical analysis, and in a difficult case begins snatching at diagnostic straws. Such an order is a reflection on the diagnostic ability of the physician who gives it and does not come from those who are really acquainted with the practice of medicine. And if that is true of the doctor who gives his order *after* the physical examination what could be said of the one who orders his laboratory work *before* he takes the history and makes the physical examination?

The remedy for this state of affairs appears to be to have the clinician order only the specific tests when they are indicated. But who shall decide when the indication has arisen? The general facts seem to be that the clinician does not know. But Rockwood says that the indications for the use of these tests are now just as definite as are those for certain other accessory procedures, and he goes on to show that the type of case in which abnormal conditions in the blood will occur can be predicted with a high degree of accuracy, and he also says it is just as much the duty of the clinician to make this preliminary prediction as it is for him to make a preliminary diagnosis of pulmonary tuberculosis from the history and physical signs before ordering a roentgenogram.

It is of course one thing to criticize and another to suggest reform. Rockwood does both. It would be going too far afield to review his article *in extenso*. It is an article which should be read and digested by every clinician and by very many clinical pathologists. But this much we can say that each blood chemical situation is discussed thoroughly enough and that from the discussions ten clinical aphorisms have been extracted. They might well occupy a prominent place in every medical office. They follow:

- 1 Never ask for both nonprotein nitrogen and urea in the same patient
- 2 Except in emergency, never ask for a nonprotein nitrogen determination when the phenolsulphonaphthalein secretion is normal. Determine the output of phenolsulphonaphthalein first
- 3 Never ask for the creatinine value of the blood unless the nonprotein nitrogen content is above 60 mg per hundred cubic centimeters. Then determine the concentration of creatinine as a matter of routine
- 4 Order determinations of the uric acid content only in cases of gout or suspected gout.

5 Order blood-sugar determinations only in cases of diabetes or suspected diabetes or hypoglycemia

6 Ask for a test of the carbon dioxide combining power of plasma in

a Diabetic patients with diacetic acid in the urine

b Uremic patients with nitrogen retention and dyspnea

c Patients showing toxic symptoms who are receiving large doses of alkali

d Conditions associated with disturbed motility of the gastrointestinal tract with marked toxemia

e Tetany of any type

7 Order chlorides, nonprotein nitrogen and carbon dioxide combining power determinations in all cases of disturbances of gastrointestinal motility with marked toxemia

8 Ask for serum bilirubin or icterus index tests in cases of jaundice, but do not pay much attention to borderline values

9 Ask for blood calcium determinations only in cases of tetany of unknown origin

10 Order inorganic phosphorus tests, if practicable, only in cases of rickets and infantile tetany

In connection with this work of Rockwood it is useful to consider an article by V C Myers. Rockwood writes from the standpoint of clinical medicine, Myers rather from that of the biochemist. One gains perspective from a perusal of both.

But also one gets from Myers' remarks an idea of how difficult it is apt to be to depend upon the work of mere technicians for accurate biochemic determinations. One is made to feel that we need more and better biochemists to oversee and evaluate the work of accurate technical assistants. So, says Myers, "although the help of technical assistants in a hospital laboratory is almost indispensable, such help is positively dangerous unless the work is under the direct supervision of some one with competent chemical training." Where does that put the blood chemical determinations of the ordinary back-room-doctor's-office technician? And beside, as Haldane says, "competent biochemists are very rare."

Well, what's the answer to it all? Thus, that the clinician should be thorough in his special work and that he should know the meaning of laboratory procedures. He should then ask his laboratory for reports on certain specific essential aspects of his cases, and not for unessential data. And he should assure himself that his highly specialized work in biochemistry is done by, or under the direct supervision of, competent persons.

#### REFERENCES

- Rockwood, Reed J. A. M. A. 91, 157, 1928  
 Myers, Victor C. Ibid. 91, 167, 1928  
 Haldane Possible Worlds, Harper's 1928, p. 83

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## News and Notes

The next annual meeting of the American Society of Clinical Pathologists will be held in Portland Oregon July 5 6 and 8 1929 All members are urged to make plans to attend this convention which besides promising a great intellectual feast will also enable them to visit our great far west

The attention of the members of the American Society of Clinical Pathologists is called to the fact that at our next convention in Portland Oregon one half day will be devoted to a group presentation and general discussion of the subject of 'Undulant Fever' Participation in this symposium by all the members is earnestly requested by the Program Committee and Research Committee

Dr H H Fosskett of Portland Chairman of the Local Committee has been making extensive preparations for the meeting and assures the members good accommodation Reservations however should be made now specifying if these should be held over for the American Medical Association Convention Those desiring same should communicate with the Secretary at once

Members will kindly report any change in address to the Secretary of the American Society of Clinical Pathologists Metropolitan Building Denver Colorado



The hospitals throughout the country are showing great interest in the work of the Registry of Technicians. Numerous requests for information have been received and also a large number of applications. The interest shown in this field of activity of the American Society of Clinical Pathologists evidences the existence of a problem which the organization has undertaken to solve.

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#### REGISTRY OF TECHNICIANS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

In view of the present chaotic condition existing among the technical workers in the clinical laboratories of clinical pathologists and hospitals and the diversity of qualifications among these workers, the American Society of Clinical Pathologists, whose members are most vitally interested in the matter, has taken upon itself the task of clarifying the situation by instituting a Board of Registry and issuing certificates to properly qualified technicians. It is the endeavor of the Society to invest this useful calling with the dignity that it deserves and to create a proper spirit of appreciation for the members of a vocation which is dedicated to the aid of suffering humanity.

There is also an urgent need for proper control and survey of schools for laboratory technicians. It is the intention of the Registry to make periodic inspection of schools and laboratories that conduct courses of training and to give certificates only to such as meet the requirements. This move is necessitated by the existence of several schools of doubtful efficiency who by promises of lucrative positions will lure ill-prepared students to take brief courses of instruction at exorbitant rates. By the system of licensing the Society hopes to benefit prospective students in affording them opportunity to seek tuition in properly qualified schools or laboratories.

Another useful feature of the Registry is the Placement Bureau for Registered Technicians which promises to be a great boon both to the laboratory as well as to the licensee.

# *The Journal of Laboratory and Clinical Medicine*

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## CLINICAL AND EXPERIMENTAL

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### LATENT TOLERANCE IN DIABETES MELLITUS A STUDY OF THE EFFECT OF HIGH SUGAR DIETS WITH INSULIN ON CONTROLLED DIABETICS\*

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By R B GIBSON, PH D, IOWA CITY IOWA

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THE administration of glucose by mouth as in the ordinary blood sugar curve test results usually in blood sugar values at the end of two hours which are lower than the fasting level. Autogenous hypoglycemic symptoms may be provoked following sugar ingestion<sup>1</sup>. When the double blood sugar curve test of Hamman and Hirschman is done on the diabetic an increase in sugar tolerance during the second period as compared with the first is observed as for the normal subject. Our surgical diabetics who are given insulin and sugar (orange juice and lactose) as preoperative preparation<sup>2</sup> withstand surgical procedures as well as or better than nondiabetic patients, when the operation is of minor character, some immediate improvement in tolerance often may be observed. Therefore, we were not unduly surprised when a patient with failing heart and edema nephritis and a diabetes mellitus (controlled) was thrown into a hypoglycemic reaction on the third day of our high sugar cardiac diet<sup>4</sup> with what we considered a safe insulin dosage for the carbohydrate given.

Accordingly it seemed to the writer that the stimulating effect of sugar ingestion on the carbohydrate tolerance might be used in diabetic management. The results have been successful beyond expectation.

Diabetic patients admitted to our service are hospitalized and placed on a maintenance diet immediately. Protein carbohydrate and fat are given in a proportion of 1:1:2.5 by weight with a fatty acid glucose ratio of 1:5. The

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From the Laboratory of Pathological Chemistry the University Hospital the State University of Iowa

Read before the American Physiological Society Ann Arbor Mich April 13 1928

Received for publication July 3 1928

energy value of the diet is increased by added fat at the time of discharge, bringing the fatty acid-glucose ratio to 20. When indicated, insulin is given in sufficient dosage to promptly desugarize the patient without change in diet. No attention is paid to ketone excretion, unless acetone and diacetic acid are present in excess. Some subsequent improvement in tolerance may occur over a period of weeks.

When the above management is interrupted by short periods (two to three days) of high sugar ingestion, with increased insulin controlled by frequent blood sugar determinations, a remarkable improvement in tolerance develops permitting a reduction in unit dosage when the regular diabetic diet is resumed. Successive periods of high sugar ingestion at intervals of four or five days may result each time in further improvement. It is possible to progress in this way until insulin therapy may be entirely withdrawn in some cases, even in young individuals. Two or three high sugar periods suffice.

With two mild diabetics, the high sugar period was not interrupted, but the insulin dosage was progressively reduced until discontinued on the eighth day. Substitutions and additions were then made to the high sugar diet. One of these patients, who is employed in the hospital, now on a general diet, has had normal blood sugar values since the experiment (six months). His diabetes had been controlled for seven months before the high sugar period was started.

The first case report is given because it demonstrates so well the improvement in tolerance resulting when a high sugar period is introduced into the established management. Though marked progress is confined to the first two or three days, this period was continued into the fifth day. It seemed possible with this patient that the tolerance would improve so much that she could carry the high sugar diet with a potential glucose value of 240 gm. on the same insulin dosage required with the diabetic diet glucose value of 100 gm. This actually happened, it is a striking demonstration of the effect of the high sugar diet. With resumption of the diabetic diet, the usual reduction of the insulin is shown.

TABLE I

## HIGH SUGAR DIET

Protein 46 gm, fat 117 gm, carbohydrate 205 gm  
Calories 2117, potential glucose 243 gm

Breakfast, 7 A M

Milk 100, cream 66, cooked cereal 120 (20 dry oatmeal), sucrose 10, orange juice 140

Dinner, 12 noon

Cream 132, butter 10, milk 100, cream 66, ice cream 100, candy 10, lettuce 50, orange 100, glucose 10

Tea, 3 P M

Milk 100, cream 66

Supper, 5 P M

Candy 20, milk 250, butter 10, aspic 100, celery 50, egg (one) 65, orange 100, glucose 20

Lunch, 8 to 9 P M

Orange 150 or apple 100

A smaller diet with calories 1978 and potential glucose of 230 gm is also used

Mrs M F, aged fifty one was admitted to the University Hospital Nov 29, 1927 with a history of diabetes of eleven years standing and with a gangrenous ulcer of the right foot from a primary infection of the toes extending over the entire foot and into the lower part of the leg. She was placed on the usual diabetic management with insulin. Incisions for drainage and removal of necrotic tissue failed to induce improvement and the right foot including the lower third of the leg was amputated December 16. At the beginning of the first sugar period, January 10 the stump had healed except for one small area which was necrotic. The patient was up and went about in a wheel chair. Her urine was free from sugar, blood sugar only slightly above normal, an equilibrium between her diet, insulin dosage, and tolerance had been maintained since December 20. Her diet consisted of protein 55 gm, carbohydrate 55 gm, and fat 137 gm, with an energy value of 1730 calories and a potential glucose of 100 gm, insulin dosage was 46 units. Blood sugar on January 6 was 133 mg two hours after breakfast. The high sugar period was started January 10 with 73 units of insulin this day. Her tolerance promptly increased so that on January 13 and 14 the insulin had been reduced to the presugar period figure of 46 units with a blood sugar figure on the fourteenth of 149 mg though the glucose value of the high sugar diet was 240 gm. The patient was then returned to the regular diabetic diet with her insulin dosage 30 units (January 16). A second high sugar period of two days duration has permitted a further reduction of insulin to 24 units daily.

## STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name Mrs M F, Age 51, Service Surgical Clinical No B 10432, Complications Arteriosclerotic gangrene of right foot Amputation 12/16/1927

NO	DATE	TOTAL URINE	SP GR	ACE TONE	DIABETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO	CAL	WT KILO	REMARKS
1	1/6	1020	1.010	-	-	-	-	133	46	55	1730		Un 20 11 14
2	7	825	1.012	-	-	-	-		46	55	1730		
3	8	850	1.018	-	-	-	-		46	55	1730		
4	9	1100	1.010	-	-	-	-		46	55	1730		Un 35 18 20
5	10	1400	1.005	-	-	-	-	137	73	H S	2113		Un 35 15 18
6	11	1800	1.009	-	-	-	-	168	58	H S	2113		Un 22 12 17
7	12	1200	1.010	-	-	-	-	155	51	H S	2113		Un 20 11 15
8	13	1025	1.010	-	-	-	-	175	46	H S	2113		
9	14	1300	1.010	-	-	-	-	149	46	H S	2113		
10	15	No		specimen		-	-	123	36	55	1730		Un 15 9 12
11	16	1450	1.016	-	-	-	-	158	30	55	1730		Un 13 7 10
12	17							155	30	55	1730		

Case reports for Mr C G and for a recently studied patient Mr D R, with the charted data illustrate the procedure which at this time seems best adapted to improve the tolerance when alternating periods of routine management and high sugar dietary are employed. These patients are young and were regarded as severe diabetics when admitted to our service. Ordinarily they would have been discharged on the insulin dosage given just prior to the time the first high sugar periods were started.

Mr C G aged twenty five was admitted to the University Hospital Jan 18 1928 with a history of diabetes mellitus beginning in May 1926 and controlled by diet and insulin by his home physician in October 1926. He complained of pain in the lower abdomen after meals thus disappeared on the third day of our routine management and the gastric analysis and series were negative. His maintenance diet consisted of protein 55 gm, carbohydrate 55 gm and fat 137 gm with an energy value of 1730 calories. A satisfactory insulin dosage 60 units daily was established January 24. During his stay in the hospital he was given four periods of high sugar diet of two days each. The third period was interrupted by a slight complication and is not given in the accompanying chart. Insulin dosage was reduced from 60 to 27 units a day after the first high sugar period, to

17 units after the second, and finally to 12 units after the fourth period. He was discharged on February 26 with an increase in his diet of 55 gm of fat, bringing the calorie value to 2240. He has reported under date of April 13 that he is doing well, has gained 7 kg in weight, and that his urine has been free from sugar.

## STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name C G, Weight 51, Age 25, Service Dr B, Clinical No C 394, Height 168

NO	DATE	TOTAL URINE	SP GR	ACF TONE	DIA CETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO	CAL	WT KILO	REMARKS
1	1/30	1500	1.010	-	-	-	-	138	60	55	1730	56.0	U 25, 15, 20
2	1/31	2000	1.012	-	-	-	-	122	60	55	1730	57.9	ditto
3	2/ 1	3000	1.010	-	-	-	-	213	71	H S	1978	59.1	U 33, 18, 20
4	2/ 2	2300	1.010	-	-	-	-	190	60	H S	1978	56.1	U 25, 15, 20
5	2/ 3	2000	1.012	-	-	-	-	133	40	55	1730	54.6	U 13, 7, 20*
6	2/ 4	1900	1.016	-	-	-	-	123	28	55	1730	51.6	U 13, 6, 9
7	2/ 5	1850	1.016	-	-	-	-	129	27	55	1730	54.5	U 12, 6, 9
8	2/ 6	1300	1.018	-	-	-	-	-	27	55	1730	53.2	ditto
9	2/ 7	2000	1.010	-	-	tr	tr	190	60	H S	1978	53.2	U 25, 15, 20
10	2/ 8	1500	1.010	-	-	-	-	182	50	H S	1978	-	U 20, 12, 18
11	2/ 9	1050	1.018	-	-	-	-	172	18	55	1730	55.5	U 8, 0, 10
12	2/10	1150	1.018	-	-	-	-	129	17	55	1730	55.5	U 10, 0, 7
13	2/11	1600	1.012	-	-	-	-	140	17	55	1730	-	ditto
14	2/12	1000	1.022	-	-	-	-	122	17	55	1730	51.1	ditto
15				-	-	-	-	-	-	-	-	-	-
16	2/19	1150	1.026	-	-	-	-	128	16	55	1730	-	U 10, 0, 6
17	2/20	900	1.020	-	-	-	-	149	14	55	1730	53.9	U 8, 0, 6
18	2/21	1500	1.012	-	-	-	-	133	55	H S	1978	-	U 25, 12, 18
19	2/22	1600	1.010	-	-	-	-	150	42	H S	1978	54.0	U 15, 10, 14
20	2/23	925	1.018	-	-	-	-	145	12	55	1730	54.0	U 7, 0, 5
21	2/24	1600	1.016	-	-	-	-	155	12	55	1730	-	U ditto
22	2/25	1050	1.024	-	-	-	-	119	12	55	1730	53.9	ditto
23	2/26			Discharged				140	12	55	2241	-	ditto

\*Given by error added orange juice to supper

Mr D R, aged eighteen, was admitted to the University Hospital May 15, 1928 for diabetes mellitus. Sugar had been found in the urine early in January of this year during an attack of mumps, though a nocturia had been noted two weeks earlier. Home treatment was dietary only, and he had lost 13.5 kg when he entered our service. Blood sugar at 5 o'clock on the afternoon of admission was 350 mg and the urine contained much

## STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name Mr D R, Weight 43.4, Age 18, Service Dr B, Clinical No C 3161, Height, 165

NO	DATE	TOTAL URINE	SP GR	ACE TONE	DIA CETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO	CAL	KILO WT	REMARKS
1	6/ 5	1800	1.010	-	-	-	-	122	30	55	1730	43.9	U 20, 0, 10
2	6/ 6	2600	1.008	-	-	-	-	155	69	H S	1978	43.9	30, 17, 22
3	6/ 7	1750	1.010	-	-	tr	tr	234	57	H S	1978	43.9	22, 15, 17
4	6/ 8	1475	1.010	-	-	-	-	105	19	55	1730	-	12, 0, 7
5	6/ 9	1150	1.014	-	-	-	-	100	17	55	1730	44.1	10, 0, 7
6	6/10	1350	1.014	-	-	-	-	127	14	55	1730	-	8, 0, 6
7	6/11	1075	1.014	-	-	-	-	130	14	55	1730	43.2	-
8	6/12	1400	1.012	-	-	-	-	96	14	55	1730	-	-
9	6/13	2200	1.010	-	-	-	-	186	60	H S	1978	-	25, 15, 20
10	6/14	2050	1.010	-	-	-	-	138	45	H S	1978	43.6	20, 10, 15
11	6/15	1600	1.012	-	-	-	-	198	5	55	1730	43.6	5, 0, 0
12	6/16	1450	1.014	-	-	-	-	172	-	55	1730	-	-
13	6/17	1300	1.012	-	-	-	-	168	-	55	1730	-	-
14	6/18	1300	1.012	-	-	-	-	162	-	55	1730	-	-
15	6/19	900	1.020	-	-	-	-	136	-	55	1730	42.7	-
16	6/20	1300	1.012	-	-	-	-	-	-	55	1730	43.4	-
17	6/21	1200	1.014	-	-	-	-	-	-	55	1730	-	-
18	6/22	1350	1.012	-	-	-	-	149	-	55	1730	43.2	-
19	6/26	1400	1.010	-	-	-	-	132	-	55	1730	43.2	-

sugar He was placed on a diet of protein 55 gm, carbohydrate 55 gm, and fat 137 gm with an energy equivalent of 1730 calories and was given insulin the following day The glycosuria was controlled on May 23 with 52 units of insulin this day Insulin was reduced gradually to 30 units on June 2 the blood sugar on June 5 being 122 mg against a blood sugar of 107 mg on the previous dosage of 37 units The first period of the high sugar diet with increased insulin on June 6 and 7 permitted a reduction of the previous 30 units to 14 units daily Insulin was discontinued after the second high sugar period, June 13 and 14 blood sugar was 198 mg on returning to the diabetic diet but fell to 136 mg on June 19 and 132 mg on June 26

The following summaries are from the records of two mild diabetics one of whom (H P W) had been controlled for six months and the other (D O'B) for a year previous to the experiment Both were given the high sugar diet and this was not interrupted Insulin was reduced daily until discontinued on the seventh and sixth days respectively Blood sugar tolerance tests on H P W were done before and after the completion of the insulin course (Table IV) Substitutions and additions were made to this patient's diet until the high carbohydrate follow up diet (Table II) was attained His improvement has been maintained and he is now after six months on a general diet The second patient D O'B was transferred after a day without insulin directly to a high carbohydrate follow up diet Except for the second day of this diet (high carbohydrate) his blood sugar remained normal and he was discharged after a week without being returned to his previous diabetic diet

Mr H P W aged twenty six weight 77 kg a medical student found sugar in his urine in the spring of 1927 As he was employed for part time in this hospital he was put on a diet prepared in our therapeutic diet kitchen and kept under observation His diabetes which was mild in type was easily controlled A blood sugar curve tolerance test on October 27 indicated that the diabetic condition was at this time even more pronounced than a previous test in June had indicated On November 13 he was given the high sugar diet The insulin dosage was controlled by blood sugar determination two hours after the three principal meals daily Twenty four hour urine collections were inconvenient and were not made but qualitative tests on specimens collected from time to time were always negative for sugar He was given 48 units of insulin (in 3 doses 20 12 and 16 units) the first day and this was progressively diminished until discontinued on November

TABLE II  
FOLLOW UP DIETS (H P W AND D O B)

Protein 83 gm fat 149 gm carbohydrate 233 gm  
Calories 2705 potential glucose 300 gm

Breakfast

Milk 100 cream 100 bread 30 butter 10 two eggs cereal (dry) 20, orange 100, sugar 20

Dinner and supper

Milk 100 cream 100 bread 30 butter 20 meat 75 potato 100 5 per cent vegetables 100  
10 per cent vegetables 100 10 per cent fruit 100

Protein 81 gm fat 121 gm carbohydrate 196 gm

Calories 2269 potential glucose 254.5 gm

Breakfast

Milk 100, cream 50 bread 30 butter 20 two eggs cereal (dry) 20 orange 200

Dinner and supper

Milk 100 cream 50 bread 30 butter 15 meat 75 potato 75 5 per cent vegetables 100 10  
per cent vegetables 100 10 per cent fruit 150

21, the eighth day of the diet. A blood sugar curve tolerance test on November 24 showed a marked improvement over the result on October 27. Blood sugar values during the insulin period varied from 100 to 155 mg, and were from 100 to 128 mg after the insulin was discontinued and the diet given a more general character.

TABLE III

EFFECT OF A HIGH SUGAR DIET WITH INSULIN ADMINISTRATION ON A MILD DIABETIC (H P W)

DATE	INSULIN	BLOOD SUGAR			DIET
		9 30 A M	2 30 P M	7 30 P M	
11/13	0 0 0	123			Diabetic diet
14	20 12 16	117	129	103	High sugar
15	15 9 10	122	112	108	
16	11 7 8	133	120	106	
17	9 5 7	145	122	103	
18	9 0 7	130	136	100	
19	7 0 0	122	155	122	
20	5 0 0	103	137	137	
21	0 0 0	125	122	122	
22	0 0 0	122			
23	0 0 0	125			
12/ 1		109			Diet more general in character, high calh and low salt (Table II)
8		117			
15		100			
30		125			
1/ 7		120			
31		126			
3/4		128			
27		127			
5/5		127			
6/24		122			General, since 5/15

TABLE IV

GLUCOSE TOLERANCE TEST ON H P W BEFORE AND AFTER THE HIGH SUGAR DIET PERIOD

HOURS	OCTOBER 27		NOVEMBER 21	
	BLOOD SUGAR MG	URINE SUGAR	BLOOD SUGAR MG	URINE SUGAR
0 0	128	none	126	none
		Glucose 50 gm		
0 5	220		179	
1 0	235	trace	179	none
1 5	197		143	
2 0	165	trace	127	none
2 5	122		120	
3 0	109	trace	111	none

Mr D O'B, aged fifty nine, was admitted to the University Hospital March 3, 1928, for diabetes mellitus. He had been managed successfully during a previous admission (May 10, 1927 to June 1, 1927) with diet alone. The diabetes was mild and had not progressed. Examination revealed only a slight secondary anemia. There was a gastric resection, which had been done two years previously, with a well functioning gastrointestinal opening. He was placed on a diabetic diet, then given two days of general diet to demonstrate the diabetic condition, after which he was returned to dietary management. The high sugar diet was started on March 14, with 44 units of insulin, and was continued with daily insulin reductions as in the case of H P W. Insulin was discontinued on March 20, when the blood sugar was 100 mg. He was then given a high carbohydrate diet of general character and was discharged a week later with a final blood sugar 110 mg.

## STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name D O'B Weight 59.9, Age 29, Service Dr F M S, Clinical No B 4221 (Ad 2)  
Height 168

NO	DATE	TOTAL URINE	SP GP	ACE TONE	DIA CETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO	CAL	KILO WT	REMARKS
1	3/6	700	1 024	-	-	-	-	108		55	1730	59.9	
2	3/7	700	1 024	-	-	-	-			55	1730		
3	3/8	300	1 024	-	-	-	-			55	1730	58.7	
4	3/9	125	1 030	tr	-	tr	tr	290		Gen.			
5	3/10	700	1 020	tr	-	tr	tr			Gen.		58.4	
6	3/11	900	1 020	-	-	-	-			55	1730	59.1	
7	3/12	900	1 012	-	-	-	-			55	1730		
8	3/13	1100	1 020	-	-	-	-	120		55	1730		
9	3/14	900	1 020	-	-	-	-	84	44	H S	1978		U 22 10, 12
10	3/15	675	1 018	-	-	-	-	120	35	H S	1978		U 10 8 12
11	3/16	1400	1 010	-	-	-	-	119	26	H S	1978		U 12, 6 8
12	3/17	750	1 018	-	-	-	-	105	16	H S	1978	58.6	U 9 0, 7
13	3/18	1450	1 012	-	-	-	-	77	12	H S	1978		U 7 0 5
14	3/19	900	1 010	-	-	-	-	106	5	H S	1978		U 5 0 0
15	3/20	1000	1 010	-	-	-	-	100		H S	1978		
16	3/21	950	1 020	-	-	-	-	108		H C	2208		
17	3/22	1350	1 012	-	-	-	-	149		H C	2208		
18	3/23	1100	1 014	-	-	-	-	104		H C	2208		
19	3/24	1200	1 020	-	-	-	-			H C	2208	60.0	
20	3/25	1450	1 014	-	-	-	-	107		H C	2208		
21	3/26	1800	1 014	-	-	-	-			H C	2208		
22	3/27	1100	1 020	-	-	-	-	110		H C	2208	59.9	

Of 19 patients who have received high sugar diets but 3 have failed to react favorably. One of these was a case of fracture of the leg with delayed healing, the second a twelve year old girl with an unstable response to usual management and the third a middle aged woman to whom the high sugar diet was given without previous control. The experiment was discontinued on one patient who failed to cooperate and on a second who developed an intestinal complication both had shown the usual improvement in tolerance after high sugar periods.

Patients whose courses can be followed and who are assured of adequate home management after leaving the hospital are now being selected for the high sugar dietary management. It is important to know how permanent the improved tolerance is and what changes in the diet may prove more efficient. Again what further increase in tolerance if any is possible for return cases previously given the high sugar diet?

It is difficult to correlate the results with current work in diabetic management and the action of insulin. Sansum Blatherwick and Bowden's diabetic control and Baumbridge's study on rats both indicate that insulin is much more effective when a diet high in carbohydrates is employed as compared with a high fat ration. Macleod<sup>7</sup> has suggested a stimulation of insulin production by glucose acting on the islet cells not yet involved in the disease process. Chabrier and Copeman<sup>8</sup> have attempted to treat diabetes with relatively high carbohydrate diets and large doses of insulin when the patient has been desugared for fifteen days and blood sugar normal, the injections may be suspended pending a return of further symptoms, when another series is undertaken. The free intervals between series were found to increase gradually with progressively diminished insulin



periods Sweeny's<sup>9</sup> recent experiments on the effect of diet on sugar tolerance are of interest in showing that diets high in fat and protein and low in carbohydrate result in lowered sugar tolerance. Latent tolerance may be due to a fixation of the sugar mechanism at a low level unless stimulated by sugar ingestion, a condition which is exaggerated in diabetes and continued, though at a different level, in the controlled diabetic. The idea that a dormant rather than a functionally inadequate mechanism may obtain in diabetes and that it may be stimulated to activity under appropriate conditions suggests itself. This is in accord with the fact that in a considerable proportion of cases no pathologic changes can be found in the pancreas (Adami and McCrae).

#### SUMMARY

Diabetics controlled on maintenance diets with a fatty acid, potential glucose ratio of 1.5 and the required insulin dosage have shown a remarkable improvement in tolerance following two or three days of high sugar ingestion with increased insulin. Successive periods of high sugar diet at intervals of four or five days result usually in a progressive increase in tolerance until dietary management without insulin may suffice to control the diabetes, especially in young individuals. Two mild diabetics, previously controlled, continued to improve in tolerance without interruption of the high sugar diet except for substitutions and additions until a diet of general character was attained.

The insulin used in this study was in part supplied through the courtesy of E. R. Squibb & Sons.

#### REFERENCES

- <sup>1</sup>Gibson, R. B., and Larimer, R. N. Hypoglycemic Symptoms Provoked by Repeated Glucose Ingestion in a Case of Renal Diabetes, *Jour Am Med Assn*, 1924, lxxxii, 468.
- <sup>2</sup>Hanuman, L., and Hirschman, I. I. Studies on Blood Sugar. IV. Effects upon the Blood Sugar of Repeated Ingestion of Glucose, *Bull Johns Hopkins Hosp*, 1919, xxx, 306.
- <sup>3</sup>Gibson, R. B. Effects of Insulin and Glucose on Acid base Balance in Surgical Diabetic Patients, *Jour Am Med Assn*, 1926, lxxxvi, 1209.
- <sup>4</sup>Smith, F. M., Gibson, R. B., and Ross, N. G. Diet in the Treatment of Cardiac Failure, *Jour Am Med Assn*, 1927, lxxxviii, 1943.
- <sup>5</sup>Sansum, W. D., Blatherwick, N. R., and Bowden, R. The Use of High Carbohydrate Diets in the Treatment of Diabetes Mellitus, *Jour Am Med Assn*, 1926, lxxxvi, 178.
- <sup>6</sup>Bainbridge, H. W. The Reduced Sensitivity to Insulin of Rats and Mice Fed on a Carbohydrate free Excess fat Diet, *Jour Physiol*, 1925, lv, 293.
- <sup>7</sup>Cited from Selle, W. A. The Effect of Glucose upon Ketosis, *Proc Soc Exper Biol and Med*, 1927, xxv, 219.
- <sup>8</sup>Chabamer, H., and Copeman, W. S. C. A New System of Treatment of Diabetes Mellitus, *Brit Med Jour*, 1926, i, 897.
- <sup>9</sup>Sweeny, M. A. Dietary Factors that Influence the Dextrose Tolerance Test, *Arch Int Med*, 1927, xl, 818.

# COLLOIDAL IRON, ELIMINATION THROUGH THE GASTROINTESTINAL CANAL\*

BY M H STREICHER, M D CHICAGO

## INTRODUCTION

A REVIEW of the literature on internal iron metabolism brings out many questions still open to discussion. Many investigations have been made on the chemistry of iron metabolism, yet no confirmation has ever been received through morphologic researches of the intestinal canal with especial reference to studies of colloidal iron.

There is still some controversy as to whether iron when given intravenously in large doses and over a long period of time is excreted with the bile, resorbed by the duodenum and later excreted in the large intestine, or whether it is resorbed by the cecum before it is eliminated in the stool.

Cushny states that iron injected intravenously into animals is stored in the liver, spleen and bone marrow, but is taken up from these organs again and is excreted by the epithelium of the cecum and colon. We were primarily interested in determining the cells that have a special affinity for colloidal iron substances in the course of elimination through the intestine.

## LITERATURE

In a careful resumé of the literature it is disappointing to note the degree of variance among different investigators as to structural components chiefly concerned in the elimination of iron and distribution of iron pigment.

Stockmann and Greig (1897) state that iron is excreted from the blood chiefly by the intestinal mucous membrane although minimal amounts are present in the bile and urine.

Beattie (1905) in his report on Hemochromatosis states that he has not found any pigment containing free iron in any part of the gastrointestinal tract.

Muir and Dunn (1914) report a positive naked eye test for iron in the mucous and muscular coats of the stomach and a negative test in the colon.

Microscopically they found few iron granules in the mucous and submucous coats of the small intestine.

Austin and Pearce (1914) state that the elimination of iron occurs almost entirely through the intestine especially the colon.

Dillard and Weidman (1925) in their report of a necropsy of a Multiple Hemorrhagic Sarcoma of Kaposi state that the colon demonstrated clusters of pigmented endothelial cells in the mucous stroma but no pigment was found in the small intestine suggesting some eliminative process in connection with this pigment.

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TABLE I  
ION OF COLLOIDAL IRON, SHOWING RELATIVE DISTRIBUTION OF IRON IN THE INTERNAL CANAL

RABBIT NO 4 20 INJECTIONS				RABBIT NO 5 20 INJECTIONS				RABBIT NO 6 60 INJECTIONS				RABBIT NO 12 30 INJECTIONS				CONTROL NO INJECTIONS RABBIT NO 7			
EL	ST	LT		FP	ST	LT		EP	ST	LT		FP	ST	LT		FP	ST	LT	
0	+	0			+	0		0	+	0		0	+	0		0	0	0	
0	0				+	0		0	0	0		0	0	0		0	0	0	
0	0				0	0		0	0	0		0	0	0		0	0	0	
0					0	0		0	0	0		0	0	0		0	0	0	
					0	+		0	0	+		0	0	0		0	0	0	
					+	+		60+	+	0		6+	0	+		0	0	0	
					0	0		0	+	0		0	0	0		0	0	0	

Pfeiffer and Boerner Patzelt claim that very few iron cells are found in the stroma of the villi of the duodenum. Many iron containing cells are found in the stroma of the mucosa of the cecum. Few iron containing cells are seen in the stroma of the mucosa of the colon. Masses of iron granules are found in the cells of the reticuloendothelial system.

#### MATERIALS AND METHODS

The data of the present investigation are based on experiments on rabbits using intravenous injections of a colloidal iron preparation. Daily injections of 2 cc of a 20 per cent solution of saccharated iron oxide were given for a period of twenty to sixty days.



Fig 1

The animals were killed by bleeding and necropsied. Sections were taken from different portions of the gastrointestinal canal, tissues fixed in 94 per cent alcohol, embedded in paraffin and stained after the Prussian blue method for iron pigment, lithium carmin being used as counterstain.

The naked eye test (Pearl Prussian blue method) was also applied in each case before fixation of tissues. The bile was collected and tested for relative amount of iron present.

#### RESULTS

Saccharated iron oxide has the same action on body tissues as vital stains have, that is iron granules are stored by the same cells that take up such dyes as lithium carmin, trypan blue etc.

In using vital stains the intensity of stain will necessarily depend upon its concentration, and therefore, we would expect to find maximum concentration in that portion of the intestinal canal where elimination is most pronounced. Table I shows the relative distribution of iron pigment in the epithelium, stroma and lymphoid tissue.

Table I demonstrates distinctly that of the intestinal epithelium, that of the cecum is chiefly concerned in the process of elimination of iron introduced in colloidal form. No pigmentation was found in the duodenum and colon. Apparently only a small amount of iron leaves the body through the bile.

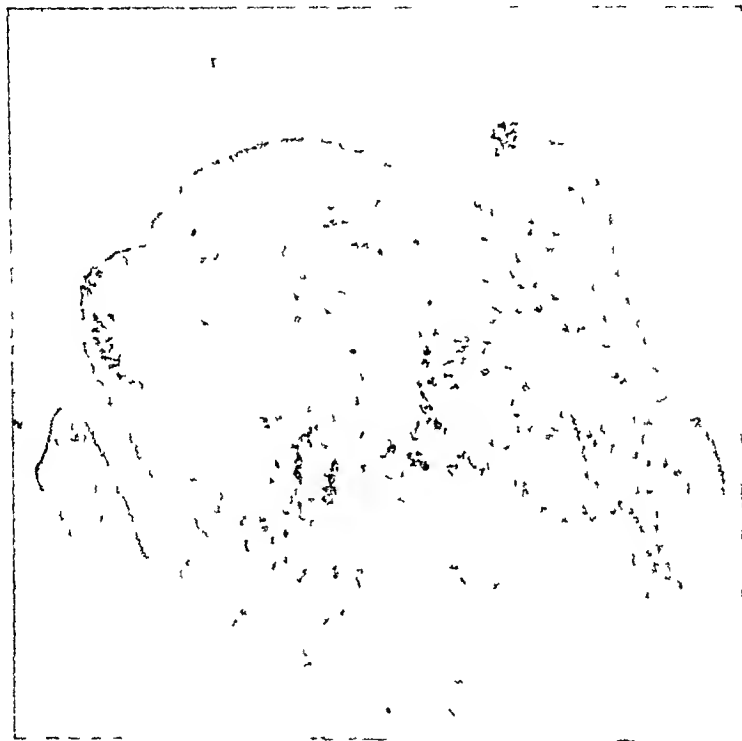


Fig. 2

The histologic analysis of the cecum may be best demonstrated by a representative photomicrograph. Fig. 1 is a photomicrograph of the cecum under low power in which the general distribution of the iron granules is shown by deposits of black particles along the lining epithelium of the cecum.

Fig. 2 is a photomicrograph of a portion of a cecum under high power in which the granules are seen to be deposited in the lining epithelium and also in the histocytes of the stroma.

#### DISCUSSION

It is a generally accepted view that certain cells will become vitally stained when colloidal metallic substances are injected into the blood stream. Eppinger was the first to show that the reticuloendothelium possessed the

property of storing colloidal iron pigment In considering the cellular elements that are specifically concerned in this process, we refer to

1 The cells of the reticuloendothelial system as in the liver, spleen, lymph glands and bone marrow

2 The liver cells and the epithelial cells of the convoluted tubules of the kidney when numerous injections are given

3 The epithelium of the cecum and the histocytes in various portions of the intestinal canal

Some investigators claim that iron when given by mouth, is first absorbed by the duodenum and later excreted in the large intestine especially by the cecum Yet, others believe that iron stored in the liver is excreted chiefly by the bile and is resorbed to some extent in the large intestine

If it is true that the iron stored in the liver is excreted in the bile, then we should expect an increase of the iron content in the bile, or if it is true that absorption is taking place in the duodenum then we should find iron granules stored in the epithelium of the mucosa, but the iron content of the bile is not increased in these experiments This is supported by Cushny who states "that iron stored in the liver does not escape by the bile" Although a small percentage of iron is a constant constituent of this fluid, it is not increased when iron is given intravenously This view is supported by Dubin and Pearce (1918), who state that in a dog, iron in appreciable amounts is not eliminated in the bile Again iron granules were not found to be stored in the mucosa of the duodenum Hence we conclude that colloidal iron given intravenously is not eliminated in the bile in large amounts and that apparently no absorption takes place in the duodenum

#### CONCLUSION

Colloidal iron given intravenously in appreciable amounts is eliminated by the cecum

#### REFERENCES

- Austin J Harold, and Pearce Richard M The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice Jour Exper Med 1914 xx, 122
- Beattie, James M Haemochromatosis, With Diabetes Mellitus Jour Path and Bacteriol, 1906 ix 117
- Dillard G J, and Weidman F D Multiple Hemorrhagic Sarcoma of Kaposi Arch Dermat and Syph 1925 xi No 2 203
- Muir Robert, and Dunn, John The Iron Content of the Organs in Bronzed Diabetes (Haemochromatosis), Jour Path and Bacteriol 1914 15 xix, 226
- Muir, Robert, and Dunn John Absorption of Iron After Haemolysis Jour Path and Bacteriol, 1915, xix 41
- Stockman R, and Greig, E D W Ingestion and Excretion of Iron in Health Jour Physiol, 1897 xxi 55
- Voigt I Beitrag zur Kenntnis der Verteilung kolloider Metalle im Sagnetierorganismus Virchow's Arch, 1925 cclvii, 8-1

# THE CLINICAL USE OF A COLLOIDAL ALUMINUM HYDROXIDE AS A GASTRIC ANTACID\*

BY BURRILL B. CROHN, M.D., NEW YORK, N. Y.

FOR the last several years internists interested in gastroenterology have felt a certain dissatisfaction with the increasing use of soluble alkaline salts to reduce or neutralize gastric acidity (hyperacidity). Laboratory tests with the fractional test meal as a medium of experimentation have shown that soluble alkalies like bicarbonate of soda, when administered by mouth, cause an immediate and total neutralization of all the gastric acidity (with a temporary suspension of digestion). This is followed by a rapid rebound of gastric acidity resulting within a half hour in an hyperacidity of even greater degree than in a control curve. These same results hold equally well in the case of the less soluble magnesia salts, such as magnesium oxide and magnesium phosphate.

In 1917 definite deleterious toxic effects were noted by Hardt and Rivers<sup>1</sup> to result from excessive dosages of soluble alkalies particularly in cases of gastroduodenal ulceration. Here the prolonged administration of such drugs in large doses, frequently given over long periods of time, resulted in definite untoward clinical states characterized by headache, malaise, anorexia, nausea and vomiting, diarrhea and marked prostration. Gastric tetany, one of the most dreaded of complications particularly in ulcer cases, while probably not caused by actual over-alkalinization, is definitely closely associated with a condition of plasma alkalosis as evidenced by the high  $\text{CO}_2$  coefficient in the plasma of such cases.

The dissatisfaction with the soluble alkalies has suggested the greater popularization and wider use of insoluble neutral salts which, dissociating in the gastric secretion, result in sufficient neutralization to give clinical relief. Such salts, containing calcium as the carbonate or phosphate of lime, are not absorbable as such from the gastrointestinal tract and are thus devoid of toxic after-effects. Greenwald<sup>2</sup> has also pointed out that the neutral secondary and tertiary phosphates of magnesium and calcium acted as local antacids without producing systemic alkalinization.

The introduction during the last few years of a special colloidal form of aluminum hydroxide as a gastric antacid has interested me as offering perhaps an optimum medium for the reduction of gastric acidity (and so-called hyperacidity), and as suggesting a salt which is devoid of any secondary acid-stimulating effect. It has also seemed desirable to administer only such a substance which, while reducing acidity to a point where clinical heartburn and pain are relieved, will yet not completely alkalinize the gastric chyme and so

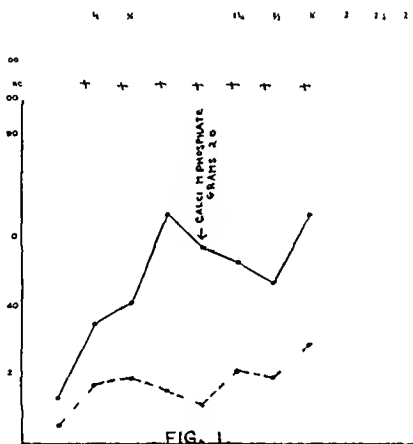
\*Colloidal aluminum hydroxide is marketed by The Wander Company of Chicago under the trade name Alucol.

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suspend digestive activity With this in mind I have completed some simple comparative studies on the effects of these latter nonabsorbable and nonsoluble salts noting their action in vivo and also their clinical possibilities and possible toxic effects

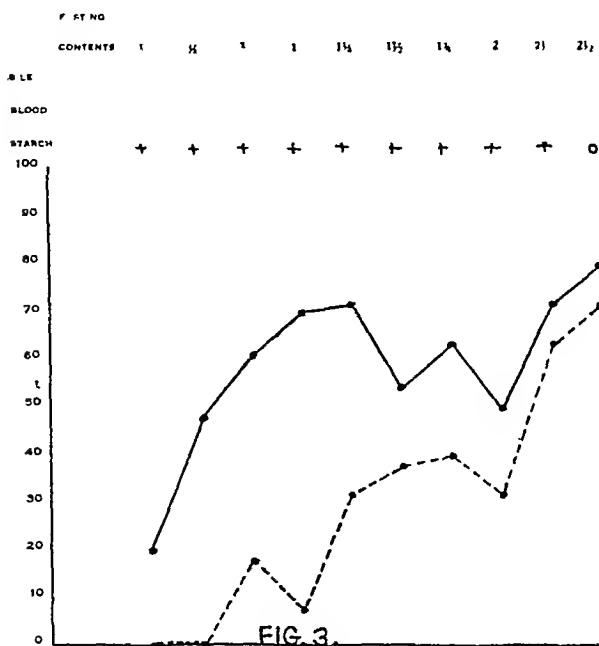
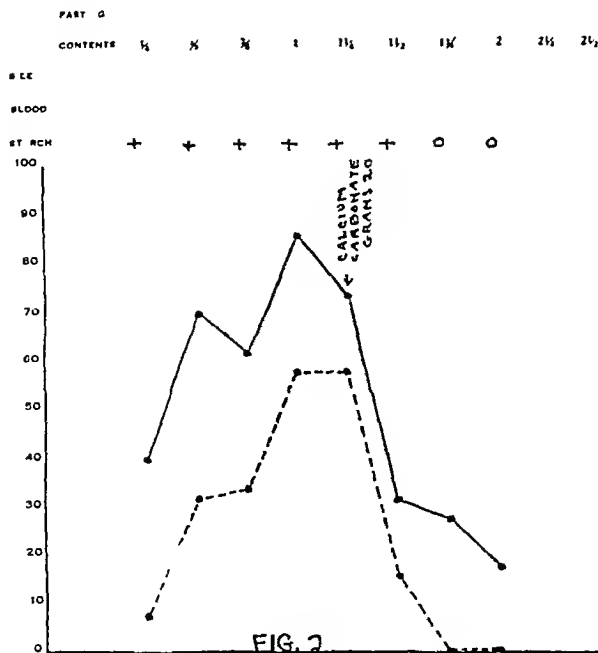
Calcium phosphate (in doses of 30 grains, 2 grams) administered to a patient with a normally high acid curve is a rather ineffectual antacid (Fig 1) Free acid is seen in the fractional test meal to be only very moderately reduced, the total acidity remaining practically unaltered

Calcium carbonate in similar dosage when administered toward the end of a test meal is more efficient as an antacid (Fig 2) but causes, like the soluble sodium salts, a complete gastric neutralization



Colloidal aluminum hydroxide representing a successful and permanent colloidal suspension of aluminum hydroxide, offers many advantages It is a neutral salt, it cannot produce an alkaline reaction in the gastric chyme and is therefore incapable of suspending or paralyzing digestion it is an efficient antacid which by reducing the free acidity to a minimum consistent with continued digestion, also reduces the total acidity to a point where complete cessation of subjective symptoms is almost an invariable rule, it is non absorbable and nontoxic Fig 3 represents the control curve in a case of gastric hyperacidity with subjective symptoms of heartburn and postprandial discomfort On a successive day 30 grains of colloidal aluminum hydroxide were administered to the same patient three quarters of an hour after the test meal had been given and when the mounting acid curve showed that the conditions were similar to the day of the former control test (Fig 4) The effect of the colloidal aluminum hydroxide on the gastric curve is readily

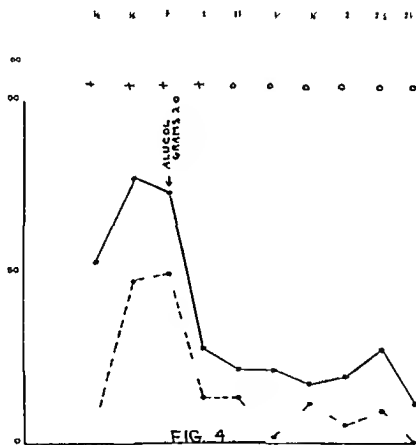




demonstrable Free and total acid are immediately reduced, and yet a minimum of free acidity is at all times retained allowing of continued digestion Motility is beneficently hastened, for while in the control curve the emptying time is two and a quarter hours, after the administration of the colloidal aluminum hydroxide emptying time of the viscus is reduced to one and one quarter hours

Results similar to the above have been found in repeated experiments both on the same individual and on several types of cases Deleterious or toxic effects have never been noted, and the action of the drug as an antacid *in vivo* is universally favorable

*Clinical Results*—I have administered colloidal aluminum hydroxide in a large series of clinical cases (fifty or more) comprising within this group



examples of functional gastric secretory disturbances of the hyperacid type complaining of heartburn, belching, discomfort and constipation, cases of gastric neurosis, and also a group consisting of gastroduodenal ulcerations In cases of functional hyperacidity and moderate subacidity with subjective complaints of pain and heartburn the relief afforded by 15 to 30 grains of colloidal aluminum hydroxide is almost immediate The relief so afforded may last for thirty to sixty minutes only occasionally being followed by recurrence of heartburn or the subsidence of symptoms may be complete and enduring An undesirable constipating effect such as is seen with the bismuth salts has not been observed, nor have nausea, vomiting or diarrhea or any other toxic by effects been noted on any occasion

In the series of over 20 cases of gastroduodenal ulceration colloidal aluminum hydroxide serves all the purposes of an ideal antacid in giving immediate

relief to the symptoms of pain and heartburn. The pain of ulcer ceases almost immediately after the colloidal hydroxide of aluminum has been swallowed. In the less severe cases the relief endures until the next meal. In the more severe cases the relief is just as efficient and prompt, but a recurrence of the pain may not be prevented after some time. By thus giving prompt and continued relief to the symptoms of pain and heartburn it allows of a more gradual and steady increase of the diet and thus probably to more ideal conditions for a rapid healing of ulcer.

To sum up, it may be said that colloidal aluminum hydroxide seems to be the more desirable of the neutral nonabsorbable antacid salts in so far as it is an efficient agent in reducing gastric acidity to a point where symptoms are relieved but gastric digestion allowed to continue. It hastens gastric emptying, it is nontoxic and devoid of deleterious by-effects. It is clinically applicable in cases of gastric secretory disturbances characterized by hyperacidity and can be used in ulcer cases in moderate dosage over prolonged periods without the anxiety of producing or the production of alkalosis or the toxic symptoms such as may be due to the absorption of soluble alkaline salts.

#### REFERENCES

- Hardt and Rivers Arch Int Med 31 171, 1923  
Greenwald Proc Soc Exp Med and Biol 20 436, 1923

1075 PARK AVENUE

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## IMMUNIZATION TO SCARLET FEVER OF PROVIDENCE CITY HOSPITAL STUDENT NURSES\*

By EDWIN M. KNIGHTS, M.S., PROVIDENCE, R. I.

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IN JANUARY, 1927, the laboratory of the Providence City Hospital extended its research work on scarlet fever to include the Dick testing of student nurses and the immunization of a certain percentage of nurses showing a positive Dick test. The results here tabulated cover the period from January 1 to December 31, 1927. The work of immunizing the student nurses is being continued this year, but for reasons to be explained later the Dick testing has been discontinued and only the immunization work is being carried on.

Before the work was started in 1927 an investigation of the methods used in the production of scarlet fever streptococcus toxins and antitoxins by various commercial biologic laboratories was carried out. We were able to obtain from one of these commercial laboratories a written statement to the effect that their scarlet fever streptococcus toxins and antitoxins were produced from pooled strains of hemolytic streptococci, some of which were subcultures of the strains used by the Dick in producing experimental scarlet fever in human beings. In addition certain strains of hemolytic strepto-

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\*From the Laboratories of the City Hospital Providence R. I.  
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cocci were used which were obtained from scarlet fever patients, but which had not definitely been shown to have caused scarlet fever in human beings. The products of this one commercial biologic laboratory have been used exclusively in this work.

The care of scarlet fever patients comprises part of the training of the student nurses at the Providence City Hospital, and during their stay here the students may be considered to be more or less equally exposed to scarlet fever. During the year 1927 there were 274 student nurses in training at this hospital. The period of training here may be either three or four months and there are no set dates for the arrival and departure of large groups of nurses. It might be said that students are arriving continually and leaving as regularly, having completed their period of training. Of these 274 student nurses 196 were given Dick tests for susceptibility to scarlet fever using 0.1 cc. of standardized toxin intracutaneously, the results being read after twenty hours.

Of the 196 nurses tested there were 53 who gave a history of having previously had scarlet fever. The results of the Dick test on these 53 were 11 positive and 42 negative. There were 143 nurses who gave no previous history of scarlet fever and the results of their tests were 37 positive, 101 negative and 5 doubtful. There were in all 48 nurses or 24.5 per cent of those tested who gave a positive Dick test and 143 or 72.9 per cent who gave a negative test and 5 nurses or approximately 2.5 per cent who gave doubtful reactions. Twenty-six of the nurses who gave positive Dick tests and who gave no previous history of having had scarlet fever were actively immunized against scarlet fever by the method of giving five injections of scarlet fever streptococcus toxin at weekly intervals, beginning with the first injection containing five hundred skin test doses of toxin and increasing the amount of toxin on each successive dose until the fifth and last dose contained 60,000 skin test doses. Not one of the twenty-six nurses thus immunized contracted scarlet fever during her period of training at this hospital. We have no record of their histories subsequent to the completion of their training here.

Fifteen of the 274 student nurses contracted scarlet fever during their course of training in 1927. None of these 15 nurses had been previously immunized but Dick tests had previously been done on eight of them. Four had given positive tests, 3 had given negative tests and one gave a doubtful test. One of the fifteen nurses contracting scarlet fever in 1927 gave a history of having had scarlet fever in 1917. Her Dick test previous to the onset of the disease had been positive and a retest made after convalescence was negative.

The twenty-six nurses who were immunized against scarlet fever were, with one exception, given a retest two weeks after their last inoculation. The results of this retest were: twenty gave negative tests, four gave positive tests and one gave a doubtful test.

During the year we were furnished with one lot of skin test toxin which when used in the regular way seemed to be giving too high a percentage of negative results. A fresh lot of toxin was obtained and this lot gave results at variance with the previous tests when repeated on the same nurses. The

commercial biologic laboratory which supplied us with material for this work has, during the present year, been unable to supply us with scarlet fever streptococcus toxin standardized for skin test work. In a letter to us they state, "We have shipped no Dick test for the past month. About the middle of January, all our stock was called back to the laboratory. It will be released at a time when laboratory tests prove satisfactory."

In summarizing the results obtained during the year 1927 we may draw two conclusions:

1 The Dick test toxin with which we were furnished during 1927, gave unsatisfactory results as an indicator of susceptibility to scarlet fever.

2 While the number of nurses who were given the immunizing doses of scarlet fever streptococcus toxin is rather small, the results seem encouraging enough to warrant continuing, during the coming year, the immunization of nurses who give no history of having had scarlet fever, leaving a certain percentage not immunized for controls.

#### DICK TESTS AND IMMUNIZATION OF NURSES, 1927

Number of nurses who served part or all of their training period in 1927	274
Number of nurses tested for susceptibility to scarlet fever	196
Number of nurses with positive Dick tests	48
Number of nurses with negative Dick tests	143
Number of nurses with doubtful Dick tests	5
Number of the 274 developing scarlet fever	15
Number of nurses developing scarlet fever with previous positive Dick test	4
Number of nurses developing scarlet fever with previous negative Dick test	3
Number of nurses developing scarlet fever with previous doubtful test	1
Number of nurses developing scarlet fever not previously tested	7
Number of nurses immunized (5 doses) (one received only 3 doses)	26
Number of nurses with positive Re Dick after immunization	4
Number of nurses with negative Re Dick after immunization	20
Number of nurses not tested after immunization	1
Number of nurses with doubtful test after immunization	1
None developed scarlet fever who had previously received active immunization	

#### Dick Tests

	POS	NEG	DOUBTFUL	TOTAL
Nurses with previous history of scarlet fever	11	42	0	53
Nurses with no previous history of scarlet fever	37	101	5	143

## SECONDARY FACTORS IN UNCOMPLICATED CASES OF SEASONAL HAY FEVER

BASED ON A STUDY OF SEVEN HUNDRED NINETEEN CASES

BY RAY M. BALLYEAT, M.A., M.D., F.A.C.P., OKLAHOMA CITY, OKLA.

**D**URING the first seven years of our study of allergic diseases uncomplicated cases of seasonal hay fever were tested only with pollen. During the latter part of this period, however, we became suspicious, on account of failures in obtaining good results in some cases, that some seasonal hay fever patients might be hypersensitive to other atopic substances than pollen which would act as secondary factors. For this reason cases obtaining poor results were rechecked for the possibility of sensitivity to animal dander, other dusts, and foods. During the past two years all cases of seasonal hay fever who manifested no symptoms out of the pollen season were tested both by the scratch and intradermal methods with pollen, animal epithelial, other common inhalants, and some of the important foods just as we routinely do cases of perennial hay fever.

Seven hundred and nineteen private uncomplicated seasonal hay fever cases which have been investigated in our Clinic during the past two years comprise our present study. Our findings relative to a sensitivity to inhalants other than pollen in these cases interested us since they had no symptoms out of season. We believe that our knowledge of the findings enhanced the value of our management very materially, which has encouraged this presentation.

### POLLEN THE SOLE CAUSE

Of the 719 cases studied, 345, or 47.9 per cent, were not found sensitive by either method of testing to animal dander, other common dusts or rye root, eggs, milk, and wheat.

### POLLEN AS A SECONDARY FACTOR

One hundred and twelve cases of seasonal hay fever which had no symptoms prior to August 12 were studied, all of which showed a marked reaction to one or all of the three ragweeds. Of this group, 57, or 50.8 per cent, showed a marked reaction to some group of pollens other than the *Compositae* family (the group to which the ragweeds belong). In other words it appears that half of the cases sensitive to ragweed which have no symptoms until the season of ragweed bloom showed a skin reaction that was markedly positive to pollen of other groups. While these patients have not been tested by the ophthalmic test, yet, in my judgment, in the majority of cases they would be found to have a sensitive mucous membrane to correspond with the skin sensitivity. It occurred to us a few years ago that the reason why poor results were obtained in the treatment of some of the seasonal hay fever cases

was due to the fact that the other pollens to which they are sensitive might be playing a secondary part. In other words, a patient might be rather markedly sensitive to one or all three of the ragweeds and yet have no symptoms until after the ragweed season. It is very difficult to desensitize a ragweed patient sufficiently to protect him against the pollen content of the air on the few days during the season when it is exceptionally high. On these days the mucous membrane of the nose will be badly irritated and from that time on the ragweed pollen would play a very definite part in keeping up symptoms. In other words, after the nose has been injured by the ragweed pollen it is an abnormal membrane, so to speak, and then even mechanical dusts and chemical odors and the cool air will irritate it sufficiently to produce at times marked symptoms. Believing our idea just mentioned to be a correct one, during the last two years we treated a number of seasonal hay fever cases which were sensitive to ragweeds, which were also sensitive to a group of pollens other than ragweeds, but whose symptoms did not appear until the ragweed season of bloom. These patients were treated both with the ragweed pollen extract and an extract from another group of pollens, to which they were found specifically sensitive.

CASE 1—Dr J. A. H., forty-eight years of age, came to the Clinic complaining of seasonal hay fever of six years' duration. He had been desensitized two seasons prior to coming to us by using a ragweed extract and through the guidance of a most excellent man doing allergic work.

On testing him he was found sensitive to the following

Giant ragweed	++++
Short ragweed	++++
Western ragweed	++++
Bermuda	++++

He had never manifested symptoms until the fifteenth day of August. I suggested to him the possibility of Bermuda grass being a definite factor after the ragweed season appeared, and outlined his treatment accordingly, namely, using both the ragweed extract and Bermuda, but not mixed, of course, starting the treatment at such a time that both products would be built up to a maximum dose before the fifteenth of August, and excellent results were obtained. This has been repeated two years since with similar results.

It is very common with us to find cases of seasonal hay fever without symptoms until August 15, extremely sensitive to one or more of the amarantths or one or more of the grasses. In these cases we use routinely in treatment both ragweeds and the amarantths, or both ragweeds and grasses, or in some cases sensitive to ragweeds, the amaranth family and the grasses, we use all three groups. We feel that by so doing our results have been materially better.

#### RÔLE PLAYED BY ANIMAL DANDER IN UNCOMPLICATED SEASONAL HAY FEVER

A patient born with the ability to become sensitive to pollen and having seasonal hay fever theoretically should become sensitive to other atopic substances, especially if the familial tendency is great. Our findings lead us to believe that this is true since out of the 719 cases under consideration, 254, or 35.3 per cent, gave marked skin reactions to animal dander. A patient who suffers from seasonal hay fever frequently complains of the irritation of cold

air, burned bacon, smoke of any kind, etc. In other words, after the nasal mucous membrane has been made irritable by the pollen to which he is specifically sensitive, even nonspecific substances have the ability to produce an outpouring of mucus. If this be true, then it seems much more likely that patients with seasonal hay fever who are also sensitive to animal dander, would have their nasal mucous membrane irritated by the animal dander during the pollen season. It is my opinion that the animal dander to which seasonal hay fever patients are sensitive may cause no symptoms out of season, but after the nose has been irritated by the pollen, the animal dander then becomes a very definite factor.

CASE 2—Mrs B W M, aged forty two years, complained of seasonal hay fever for a period of two years.

On testing a marked sensitivity to the following was found:

Giant ragweed	++++
Short ragweed	+++
Goose feathers	++++
Duck feathers	+++

Pollen therapy was instituted with only fair results. We then elicited a history of sneezing at night, and she was advised to eliminate feather pillows from her bedroom.

The next season pollen therapy was instituted with the elimination of feather pillows from her bedroom, with excellent results.

We have had similar experiences in many other cases, which leads me to believe that feathers are a common secondary factor in seasonal hay fever and that therefore feather extract should be used routinely in testing seasonal hay fever patients. If they are found sensitive to feathers, feathers should be removed from the bedroom at least during the pollen season.

#### ORRIS ROOT AS A SECONDARY FACTOR IN SEASONAL HAY FEVER

It is not uncommon to find seasonal hay fever patients who show a marked skin reaction to orris root but have only slight or no nasal symptoms outside the pollen season. Of the 719 cases 75, or 10.4 per cent, were found to have a skin strongly positive to orris root by testing. It is only reasonable to believe that seasonal hay fever patients who show a positive reaction to orris root and pollen but who have no symptoms relative to orris root out of season should have orris root symptoms after the season appears and the nose has been made irritable by the pollen. Our own experience in many cases has proved the truthfulness of this assumption.

CASE 3—Miss L A, aged twenty four complained of seasonal hay fever of three years' duration beginning September 1 with no symptoms at any other time of the year.

On testing she was found sensitive to the following proteins:

Giant ragweed	++
Short ragweed	+++
Western ragweed	++++
Orris root	+++

Desensitizing with the three ragweeds was done with about 50 per cent relief. She had been advised to eliminate her orris root, which she had not done. After poor results were obtained during the season the orris root was finally eliminated with almost immediate relief.



This patient is one of many whom we have seen, most of them females, who are sensitive to both pollen and orris root, on whom we obtained poor results with pollen therapy alone and good results when both orris root and the pollens were used in desensitizing, or when orris root was thoroughly eliminated during the season. If a patient has mild symptoms due to a sensitivity to orris root, it is the author's judgment that desensitizing with orris root should be done along with pollen therapy, and it should be instituted prior to the season, at such a time that the maximum dose of orris root can be reached before the patient's hay fever season.

#### SEASONAL HAY FEVER CASES ALSO SENSITIVE TO FOOD

Forty-four, or 61 per cent, of the 719 cases of pollen seasonal hay fever were very definitely sensitive to one or more food proteins. Of these cases a number were treated without eliminating the foods to which they were specifically sensitive, with unsatisfactory results, only to find the results much better on eliminating the foods, either during the same season or the following season.

CASE 4—V L H, a girl, aged six, complained of seasonal hay fever of a very severe type, beginning August 15. No symptoms relative to the respiratory tract appeared prior to this time. She had eczema which had existed since infancy, however. Desensitizing had been done without relief.

Desensitizing, along with the removal of the foods from the diet, not only relieved her hay fever but eliminated her eczema.

Until the last two or three years it was generally believed that asthmatic or seasonal hay fever cases, sensitive to foods but without evidence of urticaria or eczema, should be desensitized with, or should avoid, the inhalants to which they were sensitive, but removal of the foods to which they were sensitive was not considered important. We have seen a number of cases, however, in both seasonal hay fever and asthma, who were desensitized to, or avoided the specific inhalants, with poor results, on whom we obtained excellent relief after eliminating from the diet the foods to which they were sensitive.

Although little has been written concerning the secondary factors in seasonal hay fever, I am sure that every allergist has been impressed with the importance of such factors in seasonal hay fever. The value placed upon the secondary factor, however, probably has been underestimated. It is my judgment that the part played by secondary factors is not an uncommon cause of poor results in the treatment of seasonal hay fever.

From our observation we must conclude that seasonal hay fever patients should be routinely tested with the common animal danders and other dusts, and the common foods, along with the pollens, and that if a marked skin reaction is found to the other inhalants or foods, they should at least be eliminated during the hay fever season.

We are also led to believe that many seasonal hay fever patients require desensitization against one or more groups of pollen other than the one whose season of bloom corresponds to the period over which the patient suffers.

# THE HINTON GLYCEROL CHOLESTEROL AGGLUTINATION REACTION\*

## MODIFICATIONS IN TECHNIC (Second Communication)

BY WILLIAM A. HINTON AND GENEVIEVE O. STUART † BOSTON, MASS

IN JUNE, 1927, a new serum test for syphilis was described and the results of its application on 138 syphilitics and 368 nonsyphilitics reported<sup>1</sup>

Upon further use of the test, unforeseen difficulties arose. During the summer when the room temperature was as high as 32° C it was troublesome to keep the bath at 27° C for the necessary period of incubation. Even when this obstacle had been overcome by the use of a specially constructed water bath, poor reactions would occur occasionally. Upon further investigation, it was found that chilling the reagents to a temperature lower than 27° C before they were mixed gave more uniform results. Notwithstanding these changes, sometimes serum from a known syphilitic which gave positive Wassermann and Kahn reactions would give a negative Hinton test.

In the hope of overcoming this difficulty, use was made of the observation that the smaller amounts of serum from some syphilitics gave a stronger reaction than the larger. Consequently all serums were titrated routinely. The smallest quantity used was decreased from 0.1 to 0.0125 cc and the largest increased from 0.3 to 0.5 cc. Serums from syphilitics thus titrated, which previously had given negative Hinton though positive Kahn and Wassermann reactions, frequently gave strongly positive reactions with the smaller amounts of serum. An analysis of several hundred serums showed that there were three main types of reaction: (1) those serums with a strong reaction in all amounts, (2) those with the strongest reaction in the largest amounts, and (3) a few with the strongest reaction in the smallest amounts. This "zone" phenomenon, therefore, appeared to be a characteristic of the reaction.

Even after the changes described above, the clearing of the suspension in the positive cases occasionally was not pronounced, and therefore the agglutination of the dispersed particles of cholesterol was incomplete. Consequently, the period of incubation was prolonged to forty hours (at 27° C as formerly) in order that the sensitiveness of the reaction might be increased and the difference between the positive and the negative reactions appear more definite. Much better results were obtained, as clinical investigation demonstrated. But such a long period of incubation was not so practical.

Accordingly, use was made of the observation that when the temperature was gradually raised to 37° C or even to 42° C, after an initial period of incubation of five or six hours at 27° C, the reaction was much more differentiated. Therefore, incubation at 37° C for a period of sixteen hours was tried and the

The phenomenon is one of agglutination and not of precipitation to which reference was made in the first communication.

†From the Division of Research and Laboratory Department of the Boston Dispensary.  
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readings were compared with those where the temperature had been 27° C and the period of incubation forty hours. The tests compared extremely well in the corresponding tubes where 0.1 c c of serum or less was used, but in those where 0.2 c c of serum or more was used, there was somewhat more clearing and therefore less differentiation between the positive serums and some of the negative serums, if clearing alone were the basis of differentiation. In the original description of the test, clearing alone was considered an indication of a positive reaction, but with the modified technique to be described, the criterion of positivity is based on the more certain factor, namely, *visible agglutination* of the dispersed particles of cholesterol. In strongly reacting serums, the particles of cholesterol will agglutinate into one or several large clumps suspended near the surface of an absolutely clear fluid, while the negative reactions are manifest by an even turbidity, or even by a distinct clearing of the fluid, but the absence of definite agglutinated masses of cholesterol.

We had often noted that clearing of the suspension, with resulting granularity, occurred frequently in the tubes containing the larger amounts of serum. This granularity, which might be mistaken for a weakly positive reaction, we were able to minimize by adjusting the proportion of muscle extract and cholesterol used in the preparation of the stock indicator. This adjustment is empirical and will be described later.

Furthermore, it was found that the optimum amounts of serum to be used with the modified technique were 0.1 c c, 0.3 c c, and 0.5 c c, rather than 0.1 c c, 0.2 c c, and 0.3 c c, as previously stated.

The technique which we use at present ensues.

The following reagents are required, and it is essential that clean, dry glassware be used in their preparation.

1. A 5 per cent solution of sodium chloride (C P) in distilled water.
2. A 50 per cent solution of glycerol, made by mixing equal parts by volume of distilled water and redistilled neutral glycerol.
3. A 0.7 per cent solution of cholesterol (Merck) in absolute alcohol. For rapid and complete solution of the cholesterol, place in an incubator at 37° C or even 56° C.
4. An ether-insoluble, alcohol-soluble extract of beef muscle, referred to as the "activating extract," and prepared as follows. Round beefsteak, freed from fat and connective tissue, is finely ground and dried for four or five days at 56° C in a large, flat dish. The dried muscle tissue is then finely pulverized with a meat grinder, using the machine's finest disc. Next, the powdered tissue is extracted four or more times with ether (U S P) in a glass-stoppered bottle, in the proportion of 1 gm of the powder to 4 c c of ether for each extraction. This process is completed when the last extract is colorless. Vigorous shaking by hand for ten minutes is required for each extraction. Finally, all the ether is poured off and the residue, which consists of the ether-insoluble constituents, is dried on filter paper. This is extracted in a glass-stoppered bottle with 95 per cent alcohol for three days at room temperature, in the proportion of one part by weight of the dried, ground residue to five volumes of 95 per cent alcohol.

5. Stock indicator, prepared by mixing 1 part of No. 4 (*the activating extract*) with 9 parts of No. 3 (the 0.7 per cent solution of cholesterol) and

allowing the mixture to stand in a glass stoppered bottle for from four to eight weeks to ripen. Frequently these proportions must be changed by the addition of more of No 3 (the cholesterol solution) to minimize granularity with nonsyphilitic serums in the test itself. Our method has been to prepare *glycerinated indicator* (No 6 below), using *stock indicator* (as prepared above) for comparison in actual tests with *glycerinated indicator* containing a smaller concentration of No 4. This has been done by taking a fraction of the stock indicator (1 part of No 4 and 9 parts of No 3) and adding to it 0.1 of a part more of No 3. From this a *glycerinated indicator* is prepared and compared with the *glycerinated indicator* made from the stock indicator in order to observe the relative amount of granularity which each shows with 10 or more known nonsyphilitic serums while the reaction obtained with an equal number of known syphilitic serums is moderately or strongly positive. This procedure is repeated, using 0.2 of a part of No 3 instead of 0.1 part, and then again by using 0.3 of a part of No 3 and so on until that proportion has been obtained which gives the minimum amount of granularity in the negative serums and the maximum number of positive reactions with syphilitic serums. The optimum proportions for most of our extracts have been 1 of the activating extract plus 10 of the 0.7 per cent cholesterol solution. The solution thus prepared is called the *adjusted stock indicator*. It appears to keep indefinitely.

This adjustment is required for accurate work.

6 *Glycerinated indicator* freshly mixed for each day's test is made as follows. One part of the *adjusted stock indicator*, composed of 0.7 per cent cholesterol and the ether insoluble alcohol soluble fraction of beefsteak muscle is pipetted into a glass stoppered graduated cylinder and 2 parts of the 5 per cent solution of sodium chloride added. The graduate is stoppered quickly and shaken vigorously for three minutes. Next 12 parts of the 5 per cent solution of sodium chloride are added and the contents shaken thoroughly. Finally, 15 parts of the 50 per cent glycerol solution are added and the whole is mixed well. It is important to follow these directions precisely.

In addition to these reagents and the glassware necessary in their preparation the following are required:

1. Test tube racks which hold 30 or 60 tubes are desirable, 10 or some multiple thereof in a row will avoid confusion.

2. Small test tubes 10 mm in diameter and 100 mm long commonly called serum tubes. It is important that the diameter of the tubes be fairly uniform not exceeding 11 mm because totally incorrect results may occur with the use of larger or smaller tubes.

3. An inactivating bath kept at a temperature of 55° C.

4. An incubator or Wassermann bath, preferably the latter kept at a temperature of 37° C or a degree lower, but the temperature should never be higher than 37° C. This should be determined by the use of an accurately standardized maximum and minimum thermometer.

The test is conducted in the following way:

1. The serums should be heated at 55° C for one half hour preferably just before they are tested.

2 With a 1 c c pipette graduated in one-tenths, pipette into the first tube 0.1 c c of serum, into the second 0.3 c c, and into the third 0.5 c c

3 With a 10 c c pipette, add 0.5 c c of the glycerinated indicator to each tube

4 Shake the rack by first inclining it so that the tubes are almost horizontal and then thrusting it quickly forward and backward. The first motion is as if one wished to throw the tubes from the rack. By repeating 10 times, one obtains a quick but thorough mixture of the serum with the glycerinated indicator. The presence of distinct foam in each tube is the only safe criterion of adequate mixing. This is an important step in the procedure.

5 Place the rack containing the tubes in a Wassermann bath or incubator, maintained at 37° C (it may be slightly lower but no higher) and let it remain for sixteen hours (conveniently from 5 P M to 9 A M). Care should be taken not to disturb the contents of the tubes during or after incubation.

6 The results should be read within an hour. Negative reactions manifest themselves by a turbidity uniformly distributed throughout the fluid or even a clearing of the fluid, but no sizable agglutinated masses. Clearing with no agglutination indicates that the temperature has been too high, or the incubation period too long, or both. Weak or moderate positive reactions show a water-clear fluid, throughout which may be suspended many small or a few large clumps of agglutinated particles of cholesterol (recorded "W" or "M"). Strong positives show from one to several large clumps near the surface of the fluid (recorded "+"). Neither opalescence occasionally encountered in serum, nor hemolysis, unless marked, has affected the accuracy of the test. Where two or more tubes show a weak reaction, the result is reported as "doubtful." With experience in technic, the percentage of doubtful reactions is very small. One must remember that a certain number of serums will give a stronger reaction in the 0.1 c c amount than in the 0.3 or 0.5 c c amounts. These are reported as positive.

The technic described above may be applied to unheated spinal fluids. The quantities of spinal fluid pipetted for each specimen are 2 c c, 1½ c c, 1 c c, 0.5 c c, and 0.1 c c. To each amount 0.5 c c of the *glycerinated indicator* prepared with the 5 per cent salt solution is added. The results should be read after a period of sixteen hours at 37° C and again after a period of twenty-four hours at room temperature, because some of the positives are manifest only after the longer period. Spinal fluids never give granular reactions unless they are positive, hence the slightest changes are interpreted as positive. We have examined 100 spinal fluids and have found that the results are practically parallel with those of the Wassermann. A detailed analysis of these results will be the subject of another communication.

#### SUMMARY

Modifications have been described in the technic of a seroagglutination reaction for syphilis which is applicable to both serums and spinal fluids.

#### REFERENCE

- 1 Hinton, William A. A Glycerol Cholesterol Precipitation Reaction in Syphilis, Boston M & S J 196 993 996, June 16, 1927

## THE QUESTION OF REFLEX VOMITING FROM THE HEART INDUCED BY DIGITALIS BODIES\*

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WHETHER digitalis induces emesis by central or peripheral action is doubtless a question which many believe was settled long ago. The matter is not so simple, however, and is yet a point of considerable importance. Lately it has been brought to the fore by Drs Hatcher and Weiss,<sup>1 2</sup> their thesis being that digitalis bodies induce emesis by stimulating afferent nerve endings in the heart. The argument is based on experiments extending over a number of years and is the outcome of much thought devoted by Prof Hatcher and associates to the subject of vomiting in its various aspects. Nevertheless, in spite of all that has been written about it, the vomiting reflex is still obscure in certain pharmacologic details as well as in other aspects. In this connection it must be said that Dr Hatcher and his colleagues have done an important service in publishing the results and conclusions from their extensive researches.

Briefly, the conclusion of Hatcher and Weiss that digitalis emesis is due to the drug's action on the heart rests upon three observations made in the Cornell Laboratory: (a) that removal of the entire gastrointestinal tract does not prevent the emesis; (b), that denervation of the heart by operative means usually does so; (c), that meotine also prevents the emesis, apparently by the paralysis of the cardiac afferent nerve endings. The idea has also had considerable support from the clinical angle in the hands of Eggleston and Wyckoff,<sup>3</sup> so that the hypothesis appears to have a well established basis.

When, a short time ago, we discovered the powerful emetic action of kstrophanthudin† and that this substance is somehow rendered inactive in the body with relative rapidity, we had a means of studying repeated emesis induced by a substance which is closely related to digitalis both pharmacologically and chemically and which has a certain advantage in the safety with which it can be used to induce emesis. As we were interested in the experiments of Hatcher and Weiss<sup>4</sup> which first led them to conclude that the heart is the locus of action in digitalis vomiting we naturally began to study the effects of strophanthidin and also true digitalis principles in animals with denervated hearts. Our results with strophanthidin were given out in 1926<sup>5a</sup> and were the first experiments to run counter to those of Hatcher and Weiss. In the meantime we have reexamined our data dealing

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†This is the noncarbohydrate portion of Kombé strophanthin obtained by hydrolysis of that body.

with emesis induced by digitalis bodies and have concluded to publish them, even though certain of our experiments have not been finished, because we are satisfied that our findings are sufficient to make it doubtful that digitalis emesis is due to action of that drug on the heart alone. That is all we attempt to show at this time.

The experiments, which have been published in detail,<sup>2b</sup> were all done on cats. While we used a large number of these animals in our first work on this problem, only twenty-two remained alive long enough to be serviceable after denervation of the heart. Sixteen of these were injected with two or more of the following substances, k-strophanthidin, ouabain, digitoxin, true digitalin, amorphous strophanthin and fluid extracts and tinctures of digitalis. In a total of forty-seven injections, either intraperitoneal or intravenous, only five failed to induce emesis. These sixteen cats had all been allowed various periods, amounting to days or weeks in some cases, for recovery from the denervation operation.\*

Briefly, this was carried out in two ways: (a) The stellate ganglia were removed by an extrapleural method and the two vagi cut in the neck, one vocal cord having been excised to prevent suffocation and the whole operation requiring about an hour and a half; (b) the denervation was accomplished in three stages, with an interval of two weeks or more between them: the left vocal cord was removed, the thorax was opened, the stellates and much of the sympathetic chain excised and the right vagus cut, the left vagus was cut in the neck and the inferior cervical ganglion removed. By this longer technique a pretty thorough denervation of the heart was accomplished, and always plenty of time for recovery from the anesthesia was allowed before injections were made.

Another group of six cats was prepared by denervating the heart at one operation, instead of in several stages, and after an hour or two allowed for recovery they were injected intravenously with ouabain in an amount known to be emetic in the normal cat. Two of the six cats vomited. Two of the four which failed to vomit died and the remaining two were given a second dose and both vomited. The results in this group of six cats were not very decisive, therefore, though they did suggest that when the period of recovery from the operation was short, emesis was interfered with by the depression incident to the operation and anesthesia.

In the light of all of these experiments we concluded that digitalis bodies (including pure principles) can induce vomiting in animals after denervation of the heart, if they are given sufficient time to recover from the immediate effects of the operation on the cardiac nerves and from the anesthesia.

Hatcher and Weiss in their papers<sup>1, 2</sup> allude to the possibility of regeneration of nerve fibers severed in the denervation of the heart in our experiments. In five of our animals that was impossible, for they were used within forty days after the operation. According to Cannon<sup>6</sup> that would be too

\*The denervation of the heart is discussed in our strophanthidin paper and in our recent article on digitalis emesis referred to above. Certain improvements in the denervation technic are necessary and these will be described in a later paper.

short a time for certain regeneration. Two others were used within sixty days. In the remaining nine, which we had to label as having a doubtful denervation of the heart, we do not know whether the individual fibers joining the cardiac plexus were afferent or efferent. We concluded, therefore, that the probability of impulses reaching the central nervous system from the heart in these nine cats was very slight.

Our results with strophanthidin have been interpreted by Hatcher and Weiss differently than by us and are regarded as supporting rather than refuting their contention that the emesis induced is of cardiac origin. As we believe that our experiments with strophanthidin have more of a bearing upon the problem of digitalis emesis than these writers are inclined to admit, we shall present the argument briefly at this point. Essentially, the reasoning of Hatcher and Weiss<sup>1</sup> is as follows:

"Suitable doses of nicotine abolish the emetic action of onabain and that of intravenous injections of strophanthidin, because nicotine paralyzes the afferent endings in the heart in which those drugs act to induce vomiting. It is possible that nicotine blocks these afferent impulses in the ganglions.

"Nicotine does not depress the vomiting center when used in this way nor does it block all paths from the periphery, and it does not have a perceptible effect on the different endings in the peritoneum, hence it does not interfere with the emetic action of an intraperitoneal injection of strophanthidin. It is clear why larger intraperitoneal doses are required after the denervation of the heart than in intact animals. After the absorption of the smaller doses from the peritoneum the drug acts on the heart of the normal cat to induce the vomiting reflex; after the cardiac nerves have been cut, such small amounts in the circulation act on the heart, but the impulses to the center are blocked and nausea or vomiting does not occur, with the larger doses however the irritant local action on the peritoneum induces vomiting."

In reply to the statements just quoted we admit that the idea of nicotine exercising a selective action as indicated is a stimulating one which furnishes a new point of view but it seems to us that the employment of that agent in the problem under discussion is not without considerable risk. Nicotine as is well known, has a complex action and Hatcher and Weiss<sup>2</sup> admit that there is a possibility of depression of the vomiting center. While their experiments seem to show that no serious depression of the sort occurred great caution is necessary in drawing conclusions as to the seat of the nicotine paralysis in the complex vomiting mechanism.

In regard to the next point which Hatcher and Weiss raise namely that larger doses of strophanthidin are necessary to induce emesis after denervation of the heart than before that operation, we must reply that the data taken from our paper on which they base the conclusion are by no means numerous enough to justify it. Neither are we ready to admit that strophanthidin induces emesis after intravenous injection because it acts upon peritoneal nerve endings. There is a possibility of such action when the emetic is injected directly into the peritoneal cavity but when it reaches the peritoneum via the blood stream after having been injected in doses of 0.10 to 0.25 of a milligram per kilogram of body weight, it must be enormously di-



luted in comparison with the concentration of the emetic given directly into the cavity when the doses mentioned are dissolved in one to five cubic centimeters of fluid

Hatcher and Weiss further suggest that inasmuch as we used hydro alcoholic solutions of strophanthidin the alcohol might be responsible for the emesis, as it is known to be irritating to the peritoneum. That is true, but we have not been restricted to alcohol as a solvent but have used acetone instead. Acetone, in concentrations which we proved would not induce vomiting when injected into the peritoneal cavity, will hold strophanthidin in solution. Such a solution will induce vomiting just as readily as the alcoholic one.

Another matter which Hatcher and Weiss stress as evidence that strophanthidin acts peripherally and not centrally to induce vomiting is its failure to act directly on the center when applied by us to the floor of the fourth ventricle. We did only a few experiments of that kind and the results, as far as they go, fall in line with those of Hatcher and Weiss who could not induce vomiting by any of the digitalis bodies they applied to the region of the vomiting center. There are various explanations which could be offered for those results, both in our experiments and in theirs, but discussion of them would lengthen this communication beyond its proper limits. We shall deal with that subject in a later paper on digitalisemesis.

For the same reason we cannot enter here into a detailed argument against the view that strophanthidin and true digitalis bodies induce emesis by action primarily upon the heart. We shall merely summarize the experimental results which we have obtained. (a) The substances just mentioned induce emesis after denervation of the heart providing that the animal is in good postoperative condition, (b) section of the spinal cord above the origin of the splanchnic nerves and below that of the phrenics does not prevent this emesis, again providing that the animal is in good postoperative condition, (c) section of both vagi does not prevent the emesis, (d) strophanthidin induces emesis in animals after denervation of the heart, no matter how it is administered, i.e., by mouth, by vein, subcutaneously, intramuscularly, and intraperitoneally. In view of these facts, it is difficult for us to conclude that any abdominal or thoracic organ is the seat of the emetic impulses. If we could cut off successfully all impulses from the abdomen and thorax and keep an animal in good condition, we might get more light on the problem. We hope to approximate that situation in future experiments.

Here we must emphasize the importance of postoperative depression as a disturbing factor in the vomiting reflex. Though Hatcher and Eggleston<sup>7</sup> recognized that any emetic may fail to act, owing to this sort of interference in a given animal, depression as a factor was minimized in the later work because, as Hatcher and Weiss<sup>1</sup> state, the far more severe operation of evisceration does not interfere with digitalis emesis. However, the evisceration experiments referred to<sup>7</sup> were done on dogs, and in their denervation work<sup>4</sup> cats were used. It does not follow that the vomiting mechanism in the one class of animals would suffer from the shock effect of an operation to the same degree as it would in another or that two operations of a quite dis-

similar character would result in the same degree of depression of the vomiting reflex in the two groups, or in either one of them

As far as we are concerned our experience with cats certainly satisfied us that the denervation of the heart, no matter how carried out, will interfere with emesis for a variable length of time, and that this result is not entirely due to the anesthesia is shown by the fact that surgical anesthesia alone, maintained with ether for a time equal to that required for a denervation operation will not prevent digitalis emesis if a few hours are allowed for recovery. We are inclined to think that division of the vagi is a thing which plays a big role and that considerable time is necessary for certain adjustments to take place in organs innervated by the vagi before the vomiting mechanism regains a normal, or approximately normal, condition. It will then react to digitalis bodies with positive nausea or emesis in nearly every case.

In closing we shall state in a few words our view regarding digitalis and strophanthidin emesis. As already mentioned, strophanthidin closely resembles digitalis bodies pharmacologically. This is shown by the marked emetic action of strophanthidin and by its action on the circulatory system<sup>8</sup>.

Doubtless the explanation of the similarity of effects is the fact of the chemical relationship which Jacobs and Hoffmann<sup>9</sup> demonstrated between strophanthidin and digitalis bodies. Our belief is therefore that all of these substances have a common seat of action in the induction of emesis and that it remains to be shown whether that seat of action if there be only one is central or peripheral.

#### SUMMARY

1 The problem of the origin of digitalis emesis is briefly reviewed and it is concluded that the theory of cardiac origin of this emesis does not rest on a secure foundation.

2 The main evidence against the view of cardiac origin is that denervation of the heart does not prevent the emetic action of any of the ordinary digitalis bodies nor of strophanthidin.

3 Since this emesis is not prevented by denervation of the heart nor by division of the spinal cord just below the phrenic nerves nor by division of both vagi, the peripheral origin of the emesis is questionable or obscure.

4 The conclusion that nicotine abolishes the emetic action of ouabain and strophanthidin and presumably of digitalis principles, by paralyzing cardiac afferent nerve endings is regarded as open to question.

5 The importance of postoperative depression as a disturbing factor in the problem is emphasized.

6 The emetic action of strophanthidin is discussed with special reference to that of the digitalis bodies. It is argued that these bodies and strophanthidin have a common seat of emetic action.

7 In the light of present knowledge based on animal experimentation it is not yet possible to say whether the emesis is of central or peripheral origin.

## REFERENCES

- 1 Hatcher, R A, and Weiss, Soma Reflex Vomiting From the Heart The Mechanism of Vomiting Induced by Digitalis Bodies, J A M A 89 429, Aug 1927
- 2 Hatcher, R A, and Weiss, Soma The Seat of the Emetic Action of the Digitalis Bodies, J Pharmacol & Exper Therap 32 37, Nov 1927
- 3 Eggleston, Carv, and Wyckoff, J The Absorption of Digitalis Bodies in Man, Arch Int Med 30 133, Aug, 1922
- 4 Hatcher, R A, and Weiss, Soma The Seat of the Emetic Action of Digitalis Bodies, Arch Int Med 29 690, May, 1922
- 5a Dresbach, M, and Waddell, K. C K Strophanthidin Emesis in Cats with Denervated Hearts, J Pharmacol & Exper Therap 27 9, Feb, 1926
- 5b Ibid 34 43, Sept, 1928
- 6 Cannon, W B Lewis, J T, and Britton, S W Studies on the Conditions of Activity in Endocrine Glands, Am J Physiol 77 326, July, 1926
- 7 Hatcher, R A, and Eggleston, Carv The Emetic Action of the Digitalis Bodies, J Pharmacol & Exper Therap 4 113, November, 1912, *ibid* 3 551
- 8 Dresbach, M, and Hosmer, Helen R The Action of K Strophanthidin on the Circulatory System, Especially the Heart, J Pharmacol & Exper Therap (See Proceed ) 27 254, April, 1926
- 9 Jacobs, Walter A and Hoffmann, Alexander A Structural Characteristic of Cardiac Poisons, Proc Soc Exper Biol & Med 23 213 December, 1925

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## THE SEDIMENTATION OF RED BLOOD CELLS\*

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SINCE Fahnaeus in 1918<sup>1</sup> revived the old observation that the red blood cells in blood obtained from patients with certain pathologic conditions settled with much greater speed than those secured from normal persons, a great literature, most of which has appeared in European journals, has accumulated upon the subject. Considerable enthusiasm has been displayed by numerous writers, while others, particularly in this country, have exhibited greater conservatism as to the value of the test in diagnosis and prognosis. Although the test appears to have considerable value in the study of certain pathologic conditions, particularly pulmonary tuberculosis, the clinical application is not the only feature to receive consideration. Interest in the test has involved primarily the mechanism whereby the rate of sedimentation is under certain conditions so markedly increased, and it is in connection with this aspect of the reaction that the following data are presented.

Almost as many theories, explaining the acceleration of the sedimentation rate in certain diseases, have been proposed as there have been authors who have reported upon the reaction. Fahnaeus thought that it was due to a change in the electric charge of the red blood cells causing a loss of their repelling force and bringing about the production and agglutinates which settled more rapidly. However, he was unable to establish that theory by experimental evidence and later suggested that viscosity may be a factor. Others such as Jevons,<sup>2</sup> Hoeber,<sup>3</sup> Risse,<sup>20</sup> and Pohle<sup>24</sup> advanced ideas similar to the theory of electric changes of Fahnaeus. Northrup and Freund,<sup>18</sup> and

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Oliver and Bernard<sup>19</sup> have shown that the stability of the red cell suspension is largely dependent upon the difference in potential between the cells and the medium in which they are suspended. If the potential difference falls to a certain point the red cells agglutinate. However, Pender<sup>9</sup> points out that calcium chloride and magnesium chloride reduce the potential difference, but the red cells do not agglutinate. A number among whom may be mentioned Katz,<sup>10</sup> Plant,<sup>23</sup> Starlinger and Flosch,<sup>34</sup> Plass and Rourke, Abderhalden,<sup>1</sup> Levinson,<sup>1</sup> Linzenmeier,<sup>14</sup> Buscher,<sup>3</sup> Westergaard,<sup>35</sup> and Alexander believe that the increased sedimentation rate is due to the increased content of fibrinogen in the blood. Newham,<sup>17</sup> however, found that the fibrinogen content was normal in 14 cases which showed accelerated sedimentation. Schmitz,<sup>33</sup> Sachs,<sup>31</sup> Salomon,<sup>3</sup> and Fischel<sup>6</sup> thought that an alteration in the globulin albumin ratio with an increase in globulin caused the increase in the rate of sedimentation. Wichels<sup>37</sup> favored the destruction of red blood cells as being the primary factor. An increase in the blood cholesterol as a cause for the acceleration was suggested by Kurten,<sup>11</sup> but this idea was opposed by Newham<sup>17</sup> who showed that the blood cholesterol was normal in 15 cases which exhibited accelerated sedimentation rates. Leendertz<sup>13</sup> believed that the presence of antibodies was the contributory factor, while Lohr,<sup>1</sup> and Herzfeld and Schinz<sup>7</sup> suggested that an alteration in the viscosity may cause the phenomenon. Others believe that the explanation does not lie in one factor alone, but that several factors collectively contribute to it. Morriss<sup>16</sup> suggests that the surface tension, capillary attraction and a difference in electric charges cause the acceleration. Pribram and Klein<sup>6</sup> think that increased viscosity and blood cholesterol may explain the reaction while Popper and Kreindler<sup>5</sup> suggest that an increase of both fibrinogen and globulin offers the explanation. Cooper<sup>4</sup> believes that an increase in fibrinogen, cholesterol and globulin is the answer and Petschacher<sup>1</sup> offers an alteration in viscosity and a high globulin content in the blood as a possible explanation. Rubin<sup>30</sup> thinks that the entire structure of the blood is altered so as to effect the characteristic acceleration in the rate of sedimentation. Puxeddu<sup>7</sup> provides evidence showing that the phenomenon is resident solely in the blood plasma while Newham<sup>17</sup> affirms that it is primarily located in the red blood cells. Wells, DeWitt and Long<sup>35</sup> have pointed out that after severe hemorrhages, the blood shows an increase in surface tension and in tuberculosis the return to normal will be slower in proportion to the progress of the disease. Other investigators have observed changes in the total protein content, in lecithin lipase and phosphorus as well as alterations in the size, number and hemoglobin content of the red blood cells of blood that shows an accelerated rate of sedimentation.

Manifestly, in view of such discordant opinions the basic cause for accelerated sedimentation is obscure.

In connection with work which has been reported on the mode of action of formaldehyde in complement fixation systems<sup>8</sup> it was observed that bacterial suspensions to which formaldehyde had been added were more difficult to throw down in the centrifuge than were those which had not been so

treated Investigation of this point revealed the fact that the addition of formaldehyde increased the surface tension and, apparently, in that way sedimentation of the bacteria was made more difficult The du Nouy surface tensiometer was employed and, in terms of dynes, the bacterial suspension showed a surface tension of 60 while the suspension with formaldehyde added to a concentration of 1:50 gave a reading of 67 dynes These facts suggested the application of the surface tension principle to a study of the sedimentation of red blood cells

The addition of formaldehyde to whole blood, to blood serum, to blood plasma, and to red corpuscles suspensions elevated the surface tension by about 5 dynes in each case even though as low a concentration as 1:250 was employed Sedimentation tests were performed after a method which has been described by Morriss<sup>16</sup> In certain tubes varying amounts of formaldehyde were mixed with the blood which was being tested Upon making readings at regular intervals, it was evident that formaldehyde altered the rate of sedimentation of the red cells A fairly strong concentration, 1:250, slowed the rate of sedimentation markedly, and weaker concentrations of formaldehyde permitted increasing rates of sedimentation up to that which was obtained when no formaldehyde was included in the test

In view of the possibility that changes other than an altered surface tension might result from the addition of formaldehyde, a surface tension reductant, sodium oleate, was utilized and a similar series of sedimentation tests were performed in which the reductant was mixed with the blood before being drawn up in the pipettes In this case it was assumed that the only changes effected by the sodium oleate were physical in character The sedimentation rate of red blood cells was very markedly reduced by the addition of minute amounts of this reductant, so markedly, in fact, that after two hours, when the control showed a sedimentation of 10 mm, the sodium oleate tubes showed none whatever This very remarkable alteration in the rate of sedimentation was interpreted as being due to the reduced surface tension By altering the surface tension with this reductant almost any degree of sedimentation from zero to that exhibited by the untreated blood may be obtained

Working on the assumption that variations in the sedimentation rate of red blood cells are due to alterations in the surface tension of the plasma, bloods were collected from patients with pulmonary tuberculosis, and sedimentation tests were conducted with and without sodium oleate, as seen in Table I

In every case the sedimentation was reduced by sodium oleate to 2 mm or less in two hours, whereas without the reductant the sedimentation was as great as 57 mm However, the protective influence against sedimentation, afforded by the sodium oleate, appeared to be lost, at least partially, after twelve hours, for a number of specimens of blood, when close to that time interval, suddenly began to sediment rapidly For example, in the interval between two and twelve hours, in one instance the cells settled from 1 mm to 60 mm, in another, from 0 mm to 45, and in still another, from 1 mm to 48 mm At the twenty-four-hour period this phenomenon was even more

marked, so that at that time the readings were nearly the same with and without sodium oleate. The explanation of this observation has not yet been obtained.

It is interesting to note in passing that the case of pulmonary tuberculosis, classified as minimal A and that classified as moderately advanced A, exhibited such slow rates of sedimentation. (In the Table they are Numbers 4 and 3)

TABLE I

TYPICAL RESULTS OF SEDIMENTATION TESTS PERFORMED UPON THE BLOODS FROM TUBERCULOUS PATIENTS AND NORMAL CASES, COMPARED WITH RESULTS OBTAINED WHEN SODIUM OLEATE WAS ADDED IN THE CONCENTRATION OF 1/2000

SPECIMEN NUMBER		SEDIMENTATION IN MM PER 100 MM HOURS					CLINICAL DIAGNOSIS
		0.5	1.0	2.0	12.0	24.0	
1	Native blood	3	15	30	46	47	Pulmonary tuberculosis far advanced A
	+ Sod oleate	0	0	0	1	4	
2	Native blood	8	24	38	47	49	Pulmonary tuberculosis far advanced B
	+ Sod oleate	0	0	0	1	43	
3	Native blood	0	0	1	5	12	Pulmonary tuberculosis moderately advanced A
	+ Sod oleate	0	0	0	1	3	
4	Native blood	1	1	2	12	22	Pulmonary tuberculosis minimal A
	+ Sod oleate	0	0	0	2	3	
5	Native blood	9	24	39	55	57	Pulmonary tuberculosis far advanced B
	+ Sod oleate	0	0	0	4	29	
6	Native blood	22	42	57	63	64	Pulmonary tuberculosis far advanced C
	+ Sod oleate	0	0	1	60	60	
7	Native blood	14	32	45	57	58	Pulmonary tuberculosis far advanced B
	+ Sod oleate	0	0	0	5	16	
8	Native blood	4	9	19	40	44	Pulmonary tuberculosis far advanced A
	+ Sod oleate	0	0	1	3	11	
9	Native blood	8	22	41	54	56	Pulmonary tuberculosis far advanced A
	+ Sod oleate	0	0	1	25	45	
10	Native blood	0	1	2	3	4	Normal
	+ Sod oleate	0	0	0	0	1	
11	Native blood	20	36	48	55	55	Pulmonary tuberculosis far advanced B
	+ Sod oleate	0	0	0	45	48	
12	Native blood	5	11	26	53	55	Pulmonary tuberculosis far advanced C
	+ Sod oleate	0	0	1	48	48	
13	Native blood	0	2	6	20	30	Normal
	+ Sod oleate	0	0	0	0	1	

It is evident, therefore, that a minute amount of sodium oleate, through its action as a surface tension reductant, very markedly decreases the rate of sedimentation of red blood cells in normal as well as in pathologic cases. In a number of instances the plasma was replaced by serum and the same rate of sedimentation was obtained in each case, which suggests that the fibrinogen factor in accelerating sedimentation is negligible.

Studies have been made upon specimens of blood from patients with pulmonary tuberculosis in varying stages, with the du Nouy surface tensiometer, but without detecting any very marked alteration in the surface tension. It is entirely possible that the changes are those of an interfacial character and not readily demonstrable by the apparatus. However, upon determining the surface tension of a specimen of plasma, it was found, for example, to be 61.7, with added sodium oleate, 52.4, and with added formaldehyde, 63.6. Nevertheless, there does not appear to be any other interpretation of the effect of sodium oleate upon the rate of sedimentation than that of an alteration of surface tension and it appears very probable that, since this reagent slows the sedimentation of blood from tuberculous patients through its effect in lowering the surface tension, the explanation of the accelerated sedimentation lies in an increased surface or interfacial tension. The cause of the alterations of the surface tension in the blood of tuberculous patients is problematical.

#### CONCLUSIONS

1 The surface tension of blood is increased by the addition of formaldehyde and is markedly decreased by the addition of sodium oleate.

2 The rate of sedimentation of red blood cells is greatly diminished by sodium oleate in normal bloods as well as in bloods obtained from patients with pulmonary tuberculosis, suggesting that the acceleration in rate of sedimentation in tuberculosis and other infectious diseases is explainable upon the basis of surface tension.

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#### REFERENCES

- <sup>1</sup>Abderhalden, E. *Munchen med Wchnschr*, 1921, *lxviii*, 973.
- <sup>2</sup>Alexander, M. E. *Med Jour and Record*, 1924, *cxxx*, 549.
- <sup>3</sup>Buscher, E. *Berl klin Wchnschr*, 1921, No 14.
- <sup>4</sup>Cooper, H. N. *JOUR LAB AND CLIN MED*, 1925, *vi*, 615.
- <sup>5</sup>Fabrics, J. *Biochem Ztschr*, 1918, *lxviii*, 375.
- <sup>6</sup>Fischel, K. *Am Rev Tuberc*, 1924, *v*, 606.
- <sup>7</sup>Herzfeld, E., and Schinz, H. R. *Strahlentherapie*, 1923, *vi*, 84.
- <sup>8</sup>Hoerber. *Klin Wchnschr*, 1922, *i*, 2412.
- <sup>9</sup>Jevons. *Tr Manchester Phil Soc*, 1870, *lxviii*.
- <sup>10</sup>Katz, G. *Ztschr f Tuberk*, 1922, *xxx*, 401.
- <sup>11</sup>Kurten, H. *Pfluger's Arch f d ges Physiol*, 1920, *clxxxv*, 248.
- <sup>12</sup>Levinson, S. A. *Am Rev Tuberc*, 1923, *vii*, 264.
- <sup>13</sup>Leendertz, G. *Deutsch Arch f klin Med*, 1921, *cxxxviii*, 234.
- <sup>14</sup>Linzenmeier. *Pfluger's Arch f d ges Physiol*, 1920, *clxxxv*, 176.
- <sup>15</sup>Lohr, W. *Mitt v d Grunzgeb d Med u Chir*, Jena, 1922, *xxxv*, 229.
- <sup>16</sup>Morriss, W. H. *Am Rev Tuberc*, 1924, *v*, 431.
- <sup>17</sup>Newham, H. B. *Quart Jour Med*, 1926, *xx*, 371.
- <sup>18</sup>Northrup, J. H., and Freund, J. *Jour Gen Physiol*, 1923, *vi*, 603.
- <sup>19</sup>Oliver, J., and Bernard, L. *Jour Gen Physiol*, 1924, *vii*, 99.
- <sup>20</sup>Pender, E. *Quart Jour Exper Physiol*, 1926, *xvii*, 173.
- <sup>21</sup>Petschacher, V. L. *Verhandl d deutsch ges f inn Med*, April, 1923.
- <sup>22</sup>Platt, E. D., and Bourke, M. D. *JOUR LAB AND CLIN MED*, 1926, *xvii*, 733.
- <sup>23</sup>Plaut, F. *Munchen med Wchnschr*, 1920, *lxvii*, 279.
- <sup>24</sup>Pohle, E. A. *Radiology*, 1925, *v*, 206.
- Popper, M., and Kreindler, F. *Paris, Med*, 1926, *lxv*, 129.

- <sup>26</sup>Pribram, H, and Klein, O *Acta Med Scandnav*, Stockholm, 1923 lvi 132  
<sup>27</sup>Puxeddu, E *Riforma med.*, 1922, xxxviii, 819  
<sup>28</sup>Reyner C E *Jour Immunol* in press  
<sup>29</sup>Risse, F *Fortschr u d Geb d Rontgenstrahlen* 1923 xxxi 148  
<sup>30</sup>Rubin E H *Am Jour Med Sc* 1927 clxxiv, 860  
<sup>31</sup>Sachs, H *Kolloid Ztschr* 1919, xxiv, 113  
<sup>32</sup>Salomon, A *Ztschr f klin. Med*, 1924, xcix, 329  
<sup>33</sup>Schmitz H, and Schmitz, H *Am Jour Obstet and Gynec*, 1926 xi 353  
<sup>34</sup>Starlinger W and Frosch, A *Med klin*, 1921, xvii, 1147  
<sup>35</sup>Wells, DeWitt and Long *The Chemistry of Blood in Tuberculosis*, Williams and Wilkins Balt 1923  
<sup>36</sup>Westergren, A *Brit Jour Tuberc* 1921, xv, 72  
<sup>37</sup>Wichels P *Ztschr f klin Med* 1925 cii 484

## THE TOXIC CONSTITUENT OF THE BILE\*

BY WILLIAM C. EMERSON, M D, DETROIT, MICHIGAN

THE fact that bile is toxic has long been observed. Bunting and Brown<sup>1</sup> in experimental work on rabbits showed that by either injecting bile intraperitoneally or by dividing the gall bladder and allowing the bile to escape into the peritoneal cavity that death occurred within twenty four hours and was attributed to direct action on the myocardium.

There has long been a controversy as to just which constituent of the bile is toxic. Bouchard, Plaesterer<sup>2</sup> and King and Stewart<sup>4</sup> all claim that the toxic constituent of the bile is the pigment. Rohrig,<sup>3</sup> Feltz and Ratter,<sup>6</sup> Leyden,<sup>7</sup> Rivosch,<sup>8</sup> Stadelmann,<sup>9</sup> and Bickel<sup>10</sup> all maintain that the toxic constituent of the bile is the bile salts sodium glycocholate and sodium taurocholate. Cholesterin and mucin surely are nontoxic.

Biedl and Krause<sup>11</sup> have shown that the bile salts are strongly hemolytic for blood and that in sufficient concentration they cause a coagulation of myosin to such an extent that the muscle loses its contractility. It is undoubtedly this action which causes death due to myocardial failure when whole bile or bile salts are absorbed by the blood over a long period of time. They also showed that when bile salts were injected intravenously a slowing of the pulse with a marked fall in the arterial blood pressure occurred.

King and Stewart<sup>4</sup> prepared their pigment from pig's bile. We repeated their work and found that their pigment when dried to constant weight equaled 85 per cent. The pigment in dog's bile equals 0.05 per cent. We would not expect pig's bile to contain one hundred sixty times as much pigment as dog's bile. It would be reasonable to assume that their pigment contained other substances. Their dried pigment was analyzed and was found to contain bile salts and large amounts of fat.

This dried pig's pigment the calcium having been removed by washing with hydrochloric acid was extracted with hot chloroform the chloroform evaporated to dryness and the residue dissolved in alcohol. This alcoholic solution of pig's pigment was injected into the femoral vein of a dog. There



was no evidence of any toxic effect Twenty-one c c of the concentrated solution, a 9 per cent solution of pigment dissolved in 95 per cent alcohol, was injected, and the blood pressure at the completion of the experiment was higher than at the beginning

Meltzer and Salant<sup>12</sup> have shown that when a few cubic centimeters of ox bile was given rapidly to a rabbit intravenously the animal died almost immediately in convulsions, while, when given slowly, as much as 50 c c of a 50 per cent solution was tolerated In all our work the material injected was given slowly 25 to 3 c c a minute

#### METHOD

Ox bile was obtained from twenty gall bladders of freshly killed animals The bile was diluted with an equal volume of normal saline and injected within three hours after collection Dogs were used in all the experiments

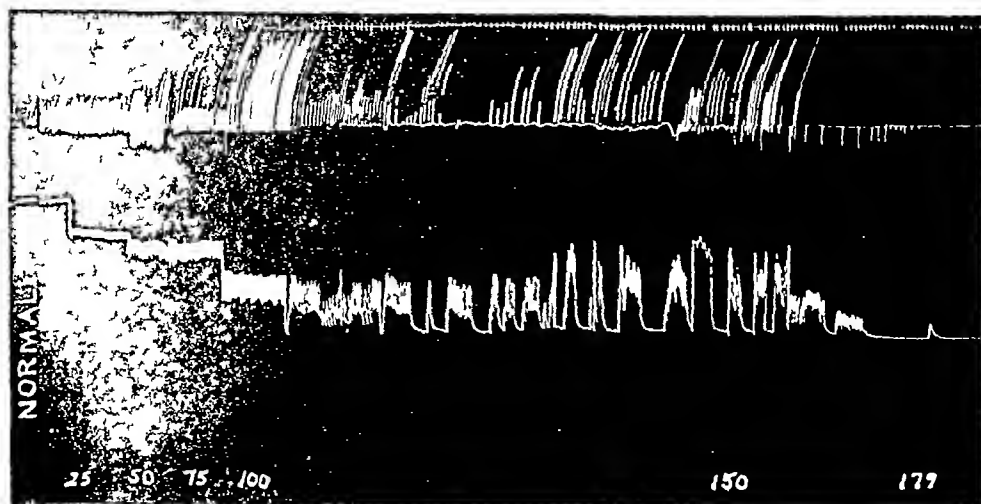


Fig 1 —Effect on the blood pressure and respiration when bile is injected into the femoral vein

The animals were anesthetized by the open drop method, later a cannula was placed in the trachea and connected with an ether bottle A cannula was placed in the carotid artery and connected with a mercury manometer for blood pressure tracing A tube was placed in the etherization system and connected with a tambour and writing lever for a tracing of the respiratory movements The femoral vein was exposed and bile diluted with an equal volume of normal saline was injected from a burette Blood pressure and respiration tracings were made after the injection of every 25 c c of the diluted bile

A 3 per cent solution of sodium glycocholate was injected into the femoral veins of ten normal dogs Blood pressure and respiration tracings were made as in the control experiments

A 3 per cent solution of sodium taurocholate was injected into the femoral veins of ten normal dogs Blood pressure and respiration tracings were made as in the control experiments

The sodium glycocholate and the sodium taurocholate were prepared as follows. The commercial product of both was obtained. This material was recrystallized three times, the final product being a pure white powder. The melting point of the sodium glycocholate was  $150^{\circ}\text{C}$  and the melting point of the sodium taurocholate was  $95^{\circ}\text{C}$ . This purified material was dissolved in distilled water and injected as described above.

On ten consecutive days ox bile from twenty freshly killed animals was obtained. The bile was diluted with an equal volume of normal saline and the mixture divided into two equal parts. One part, whole bile diluted with an equal volume of normal saline, was injected into a dog. The other half, also diluted with an equal volume of normal saline, was treated three times with blood charcoal and the final product a clear light yellow liquid, was injected into a dog. This clear light yellow liquid when compared in the colorimeter against bile diluted with an equal volume of normal saline was found to contain only 5 per cent of pigment, the remaining 95 per cent having been removed by the blood charcoal. This experiment was repeated on ten consecutive days, ten samples of bile being used and twenty dogs, two each day, injected, one with whole bile and one with bile after 95 per cent of the pigment had been removed.

Pigment obtained from human gallstones was dissolved in alcohol and injected into a dog.

#### RESULTS

Ten specimens of bile were injected into the femoral veins of ten normal dogs. The lethal dose is summarized in Table I. The lethal dose for whole bile when injected into the femoral vein gave an average of 9.6 cc per pound. Fig 1 shows the characteristic gradual fall in blood pressure which occurs when bile is injected into the femoral vein. The fall in blood pressure is later accompanied by a striking arrhythmia and at times there is an apparent cessation of cardiac activity.

TABLE I

DOG NUMBER	WEIGHT OF DOG POUNDS	LETHAL DOSE WHOLE BILE PER POUND C C
1	16.5	12.0
2	14.25	8.55
3	18.75	10.5
4	19.75	9.7
5	18	10.2
6	12.5	8.4
7	15.75	8.77
8	10.25	10.5
9	19	9.9
10	15	8.0
Average		9.6

Three per cent sodium glycocholate was injected into the femoral veins of ten normal dogs. The lethal dose is summarized in Table II. The lethal dose of 3 per cent sodium glycocholate when injected into the femoral vein gave an average of 8.5 cc per pound. Fig 2 shows the same gradual fall

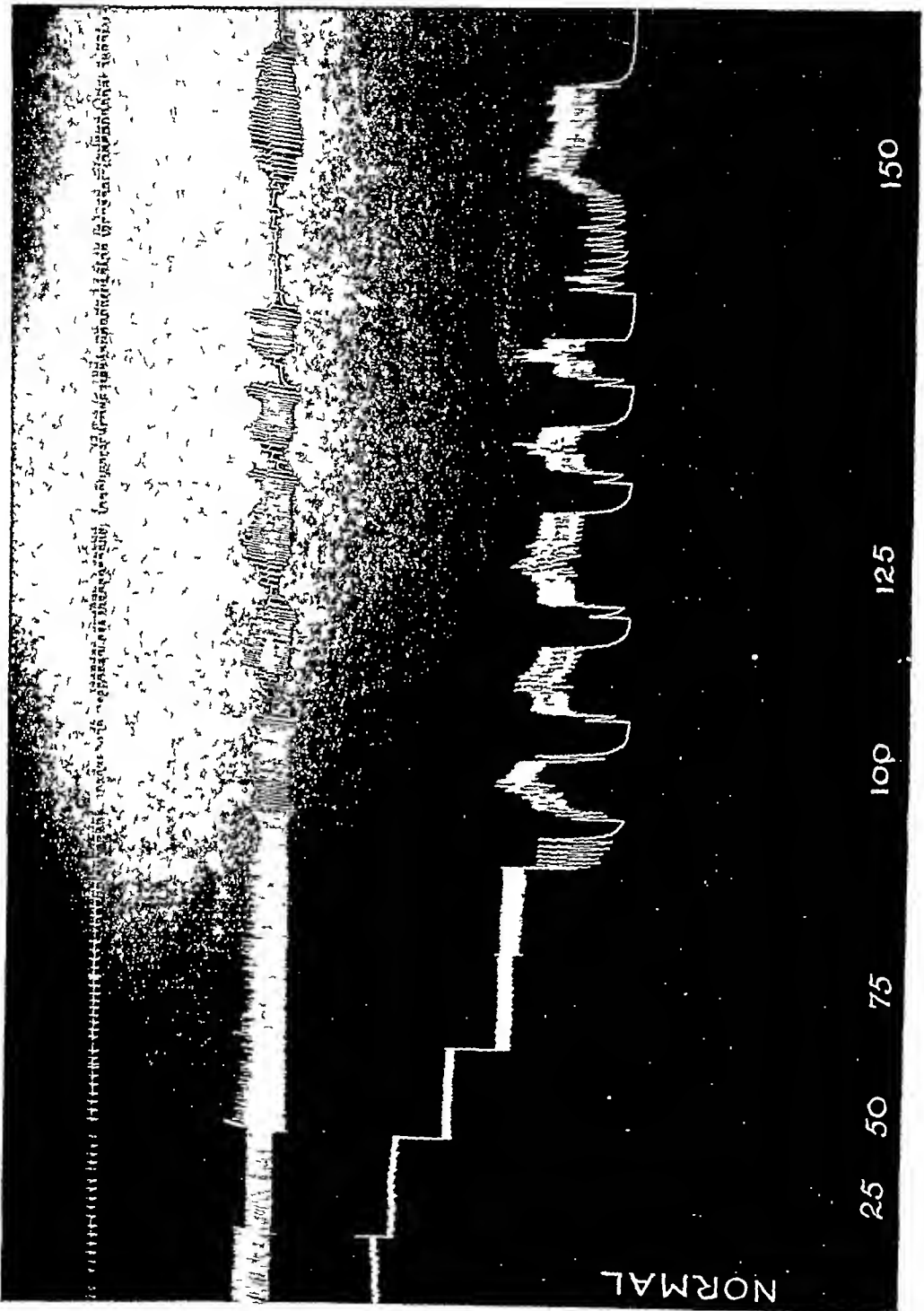


Fig 2 —Effect on the blood pressure and respiration when 3 per cent sodium glycocholate is injected into the femoral vein

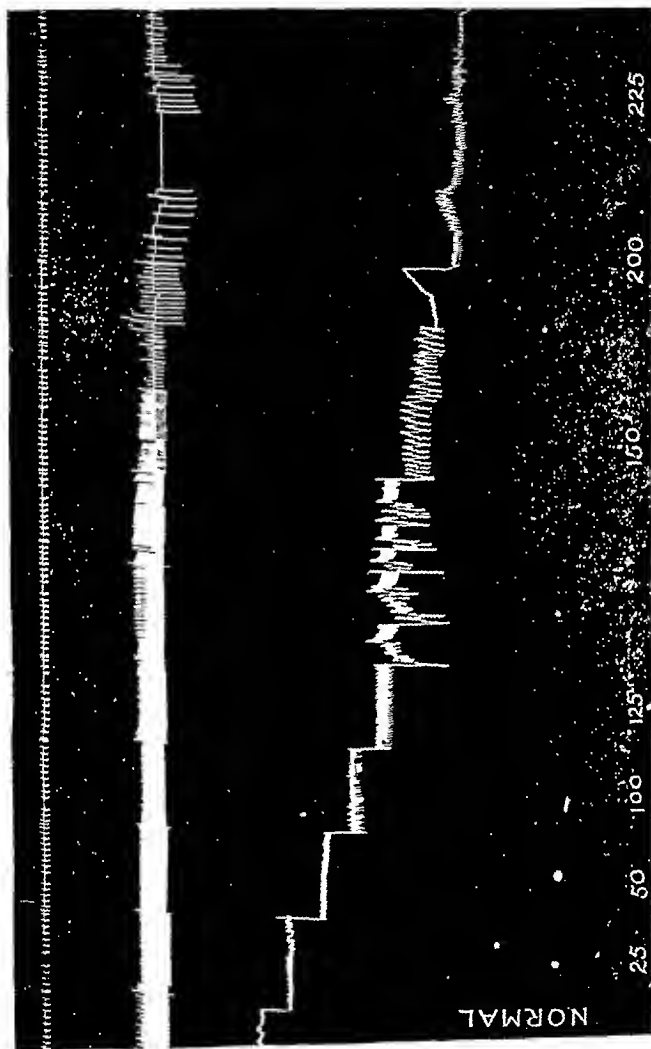


Fig. 3.—Effect on the blood pressure and respiration when 3 per cent sodium taurocholate is injected into the femoral vein

in blood pressure later accompanied by a striking arrhythmia as occurred in the control tracing when whole bile was injected

Three per cent sodium taurocholate was injected into the femoral veins of ten normal dogs. The lethal dose is summarized in Table II. The lethal dose of 3 per cent sodium taurocholate when injected into the femoral vein gave an average of 10.1 c.c. per pound. Fig. 3 shows identically the same fall in blood pressure accompanied by a marked arrhythmia as occurred in the control tracing when whole bile was injected.

TABLE II

DOG NUMBER	WEIGHT OF DOG POUNDS	LETHAL DOSE 3% SODIUM GLYCOCHOLATE PER POUND C C	DOG NUMBER	WEIGHT OF DOG POUNDS	LETHAL DOSE 3% SODIUM TAUROCHOLATE PER POUND C C
11	18.5	8.0	21	19.0	12.0
12	18.0	7.5	22	7.5	10.9
13	11.5	6.0	23	21.25	6.6
14	14.0	7.8	24	17.5	6.2
15	14.25	8.07	25	14.5	10.4
16	17.5	9.9	26	13.0	10.0
17	10.5	9.9	27	9.25	10.8
18	19.0	9.5	28	12.5	11.2
19	21.0	9.19	29	13.75	11.4
20	14.0	10.2	30	9.5	11.5
Average		8.5			10.1

TABLE III

BILE SPECIMEN	WEIGHT OF CONTROL DOG POUNDS	LETHAL DOSE WHOLE BILE PER POUND C C	WEIGHT OF SECOND DOG POUNDS	LETHAL DOSE WHOLE BILE MINUS 95 PER CENT PIG MENT PER POUND C C
1	10.5	6.2	18.0	4.6
2	10.0	7.3	20.0	10.9
3	33.0	7.8	38.0	8.2
4	7.5	6.6	9.0	10.5
5	12.5	6.7	12.0	7.3
6	26.5	9.6	11.5	9.7
7	16.5	5.7	23.0	9.4
8	14.5	5.5	14.0	10.5
9	18.5	5.7	21.0	5.4
10	17.0	6.8	23.0	7.0
Average		6.8		7.3

Ten samples of ox bile were diluted with an equal volume of normal saline. Half of each sample was treated with blood charcoal. The treated bile after 95 per cent of the pigment had been removed and the untreated bile were injected into the femoral veins of twenty normal dogs, two dogs being used for each sample of bile.

The lethal doses are summarized in Table III. The average lethal dose for the untreated bile is 6.8 c.c. per pound. The average lethal dose for the bile after 95 per cent of the pigment had been removed was 7.3 c.c. per pound.

Fig 4 shows the same fall in blood pressure accompanied later by the striking arrhythmia as occurred when whole bile was injected into the femoral vein

Pigment obtained from human gallstones dissolved in alcohol was in

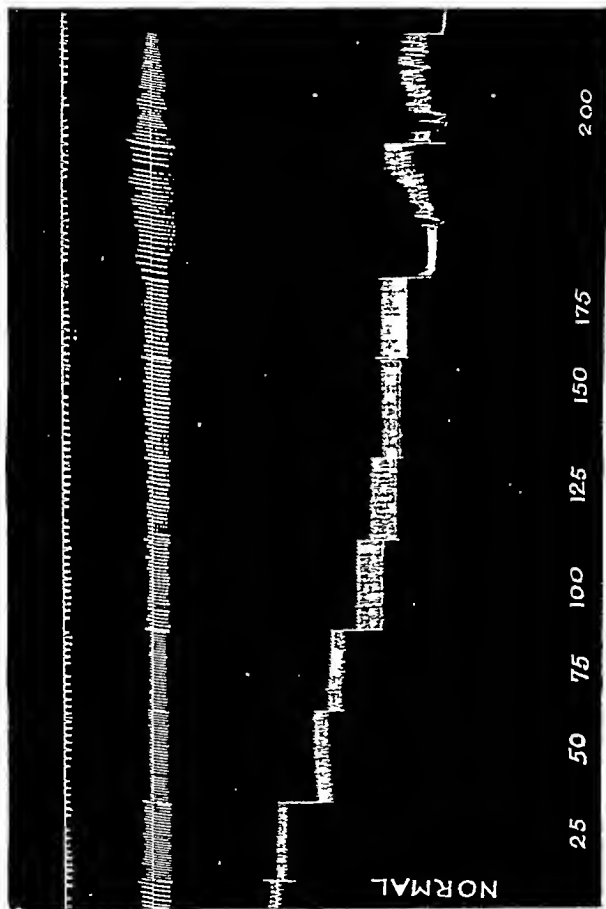


Fig 4 ---Effect on the blood pressure and respiration when bile after 95 per cent of the pigment had been removed was injected into the femoral vein

jected into the femoral vein of a dog. There was no effect on the blood pressure or respiratory rate. Twenty five cc per pound was injected, and the blood pressure was higher at the completion of the experiment than at the beginning.

## DISCUSSION

The fact that the average lethal dose of sodium glycocholate injected at a uniform rate of speed is 11 c c per pound less than the average lethal dose of bile and that the average lethal dose of sodium taurocholate injected at a uniform rate of speed is 0.5 c c per pound greater than the average lethal dose of bile would suggest that the toxic constituent of the bile is the bile salts, sodium glycocholate and sodium taurocholate.

The blood pressure and respiration tracings of the dogs injected with sodium glycocholate and sodium taurocholate being identical with the tracing of the dog injected with whole bile would further indicate that the bile salts are the toxic constituents of the bile.

Sodium glycocholate is more toxic than sodium taurocholate due to the fact that the average lethal dose of sodium glycocholate is 8.5 c c per pound whereas it required an average of 10.1 c c per pound of sodium taurocholate to kill the animals.

The average lethal dose of bile after 95 per cent of the pigment had been removed being 7.3 c c per pound as compared to an average of 6.8 c c per pound when whole bile was injected would clearly indicate that the bile pigment is not the toxic constituent of the bile.

That the bile pigment is not the toxic constituent of the bile is further indicated by the fact that the tracing of the blood pressure and respirations in a dog injected with bile after 95 per cent of the pigment had been removed is identical with the tracing when untreated or whole bile was injected, and also by the fact that when pigment was injected there was no fall in blood pressure nor was there any arrhythmia produced.

## CONCLUSIONS

- 1 The toxic constituent of the bile is the bile salts, sodium glycocholate and sodium taurocholate.
- 2 Sodium glycocholate is more toxic than sodium taurocholate.
- 3 Bile pigment is not toxic and has no effect on the blood pressure or respiratory rate when injected into the blood stream.

This work was suggested and done under the direction of Dr. Edward C. Davidson.

## REFERENCES

- <sup>1</sup>Bunting, C. H., and Brown, W. H. The Pathology of Intraperitoneal Bile Injections in the Rabbit, Jour. Exper. Med., 1911, vii, 445.
- <sup>2</sup>Bouchard, Charles. Leçons sur les auto-intoxications dans les maladies, Paris, 1887, p. 242.
- <sup>3</sup>Plaesterer, R. Ueber die giftigen Wirkungen des Bilirubins, Inaug. Diss., Würzburg, 1890.
- <sup>4</sup>King, J. H., and Stewart, H. A. The Effect of the Injection of Bile on the Circulation, Jour. Exper. Med., 1909, xi, 673.
- <sup>5</sup>Rohrig, Arch. d. Heilk., 1863.
- <sup>6</sup>Feltz, V., and Ritter, E. Action des sels biliaires sur l'économie, Jour. de l'anat. et de la physiol., 1874, x.
- <sup>7</sup>Leiden, Pathologie de l'ictère, Berlin, 1866.
- <sup>8</sup>Rykowski, D. Ueber die giftige Wirkung der Gallensäuren nebst einem Anhange ueber die Giftigkeit der Gallenfarbstoffe, Inaug. Diss., Dorpat, 1891.

- <sup>9</sup>Stadelmann, E Der Icterus und seine verschiedenen Formen, nebst Beiträgen zur Physiologie und Pathologie der Gallensecretion Stuttgart, 1891 Ueber den Kreislauf der Galle im Organismus, *Ztschr f Biol*, 1896, xvi, 1
- <sup>10</sup>Bickel, A Experimentelle Untersuchungen ueber die Pathogenese der Cholaemie, Wiesbaden 1900
- <sup>11</sup>Biedl A and Krause, R Ueber eine bisher unbekannte toxische Wirkung der Gallensauren auf das Centralnervensystem *Zentralbl f inn Med* 1898 xix, 1185
- <sup>12</sup>Meltzer S J and Salant, W Studies on the Toxicity of Bile *Jour Exper Med* 1905, vii, 280

## THE EFFECT OF A SUDDEN INCREASE IN THE INTRACARDIAC PRESSURE UPON THE FORM OF THE T WAVE OF THE ELECTROCARDIOGRAM

BY HAROLD L OTTO M D \* NEW YORK

THE effect upon the form of the electrocardiogram of a sudden rise in the intracardiac pressure within the left ventricle was investigated by clamping the thoracic or ascending portions of the aorta and in the case of the right chamber by clamping the pulmonary artery in 19 experiments on dogs† The effect of partially collapsing both the lungs or twisting the hilus of one lung was also added In order to prevent the onset of ventricular fibrillation a small dose of barium chloride (1 mg per kilogram) or ouabain ( $\frac{1}{8}$  lethal dose) was administered

In five animals, clamping the ascending aorta was followed by a great increase in the rate and diminution of the voltage of the curve In these the changes in the T wave were not considered inasmuch as they were not primary Of nine remaining animals in which the thoracic aorta was clamped, a positive influence upon the T wave appeared in five In the other four the procedure had no distinct effect upon the T wave This positive influence upon the T wave was more pronounced where a strongly negative T wave was present before In one instance (Fig 1) the effect was sufficiently intense to make the T wave upright This change in the direction of the T wave was not permanent, and the electrocardiogram slowly resumed its original form (Fig 1) The removal of the clamp was associated with the opposite effect, a negative influence upon the T wave (Fig 1 c)

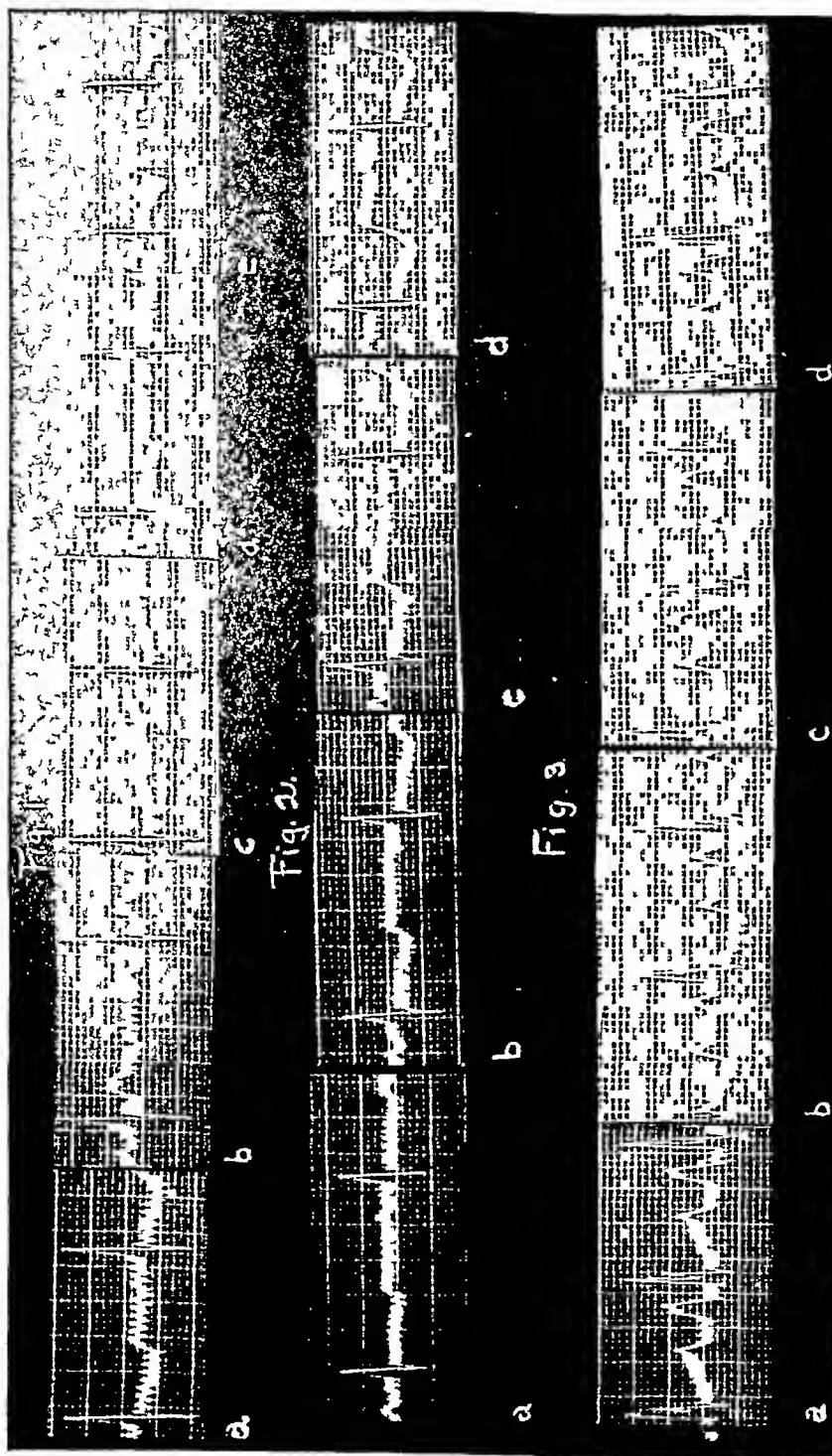
In 5 animals observations upon the effect of alterations in the pressure in the lesser circulation were made Twisting the lung hilus or incompletely clamping the pulmonary artery caused a negative effect upon the T wave A partial collapse of the lung, although giving the same result was less certain in its effect The effect here that following the clamping of the aorta was also of short duration, and the T wave slowly returned to its original form (Fig 3) When the pulmonary artery was completely occluded by the clamp the rapid death of the animal was associated with greatly exaggerated negativity of the T wave (Fig 2) The same effect occurs in association with the

From the Laboratory of Physiology Faculty of Medicine Paris

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†The animals were narcotised with chloroform the vagi cut, artificial respiration instituted and the heart exposed by the removal of the sternum Axial electrocardiograms were taken





closure of the right coronary artery<sup>1</sup> and also, circulatory failure which is induced by any procedure causing great cardiac depression

The changes in the T wave were not produced by the very slight changes in the position of the heart with reference to the line of the leading which are unavoidably associated with the procedures used. The degree to which they shifted the position of the heart was without a significant effect upon the T wave. If anatomic changes are the cause of the alteration in the T wave, they are more likely to be the effects of the slight shift in the position of the septum without alteration of the gross position of the heart which results from the changes in the intracardiac pressure.

The human electrocardiogram taken in the presence of pneumothorax often presents changes in the T wave and RT interval of the same nature. These changes are much more apt to appear with acute spontaneous pneumothorax than with the artificial type and tend to lessen as the duration of the pneumothorax increases. The experiments bearing upon the pulmonary circulation suggest that the sudden alteration of the resistance in the pulmonary circulation caused by the collapse of the lung is the cause of the T wave changes which occur in that condition.

#### REFERENCES

- 1 Otto, H. L. *Pflügers Arch f d ges Physiol* 217: 528, 1924.
- Master, A. *Am Heart J* 3: 472, 1928.

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Fig 1—Axial (RA—LL) electrocardiograms. Time in fiftieth seconds. *a* The normal electrocardiogram. *b* After application of the clamp to the descending aorta just below the diaphragm. *c* Five minutes later. *d* Ten minutes later. The T wave has resumed its original form. *e* Just after removal of the clamp.

Fig 2—Axial electrocardiograms. Time in fiftieth seconds. *a* The normal electrocardiogram. *b, c, d* Electrocardiograms at short intervals after clamping the pulmonary artery. Death followed soon after.

Fig 3—Axial electrocardiograms. Time in fiftieth seconds. *a* The normal electrocardiogram. *b* After twisting and clamping the hilus of the right lung. *c* Five minutes later. *d* Five minutes later. The T wave is again upright.

# THE ACTION OF PHOSPHATES UPON THE ENDOCRINE AND SYMPATHETIC SYSTEMS\*

By L. LOUMOS, M.D., CHICAGO

THE use of iodine in the prophylaxis and treatment of goiter and allied disturbances, also in the treatment of such diseases as, pulmonary tuberculosis, scrofula, lymphadenitis, bronchitis, asthma, etc., is well known. A great objection to the use of iodine in large doses, or in moderate doses for an extended period of time is the frequent appearance of iodism.

I have administered, in a large number of cases, iodine together with phosphates, and seldom, if ever, have I noted any symptoms of iodism so long as the phosphates were taken, therefore, the present investigation was undertaken in order to determine more definitely whether the use of phosphates increases the tolerance of iodine and prevents the manifestation of the symptoms associated with the administration of large amounts of iodine.

The fact that phosphates increase the tolerance to iodine and diminish considerably the frequency of the appearance of iodism has been noted by Huchard.<sup>1</sup> Iodine and phosphates appear antagonistic in their action, iodine being a stimulant, and phosphates depressant to the "basal metabolism activity." It was then thought advisable to investigate the action upon experimental animals.

To two dogs 0.2 gm of iodine (in tincture form) was given daily for twenty days, in addition, to one of the dogs 10 gm of equal parts of sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) and calcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ) were given daily. Both were kept on the same diet. The results are summarized in Table I.

TABLE I

INITIAL WEIGHT	TOTAL IODINE IN 20 DAYS	TOTAL PHOSPHATES IN 20 DAYS	WEIGHT AFTER 20 DAYS	REMARKS
21 lb	4.0 gm	0.0 gm	17.5 lb	First signs of hyperthyroidism on fourteenth day, then increased nervousness, ravenous appetite, etc.
20 lb	4.0 gm	20.0 gm	21.0 lb	No evidence of hyperthyroidism

TABLE II

INITIAL WEIGHT	TOTAL IODINE IN 40 DAYS	TOTAL PHOSPHATES IN 40 DAYS	WEIGHT AFTER 40 DAYS	REMARKS
18 lb	4.0 gm	0.0 gm	16.1 lb	First signs of hyperthyroidism on thirty third day. Symptoms of moderate intensity.
16 lb	4.0 gm	20.0 gm	18.0 lb	No evidence of hyperthyroidism

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The next step was to decrease the dose to 0.1 gm of iodine and 0.5 gm phosphates per day and extend the time to forty days, repeating the above experiment on another set of dogs. The results are given in Table II.

As was pointed out above, the fact that the dogs to which phosphates were administered failed to show hyperthyroidism is thought to be due to the depressing action of the latter on the basal metabolism activity. In this connection the elimination of iodine was investigated, and it was found that this is also retarded by the phosphates.

According to Kendall the normal blood iodine content is about 0.13 mg. This amount, however, varies depending upon different causes such as diet or the condition of the thyroid gland. For example, in the cretin the circulating amount of iodine is decreased. Other authors note a difference in the amount during the late summer and autumn and during the winter months.

Gley and Cheymol<sup>3</sup> conducting their investigations on goats found 0.12 mg to 0.19 mg while in our experiments on dogs (using Kendall's method) we found 0.11 mg to 0.17 mg.

A number of determinations of the iodine content of the blood with dogs taking iodine alone and also with dogs taking phosphate in addition to iodine were made and the results are summarized in Table III.

TABLE III

SPECIMEN	CONTENT OF IODINE MG PER 100 CC OF BLOOD (KENDALL'S METHOD)
1 Dog Given a total of 40 gm iodine over a period of twenty days. Hyperthyroidism. 50 cc of blood was drawn from jugular vein twenty four hours after last dose.	0.194
2 Dog Given total of 40 gm iodine and 200 gm phosphates over a period of twenty days. No evidence of hyperthyroidism. 50 cc of blood was drawn twenty four hours after last dose.	0.348
3 Dog Given 0.1 gm iodine and 10 gm phosphates daily for twenty five days. (Total 2.5 gm iodine and 25 gm phosphates). No evidence of hyperthyroidism. Analysis of blood Before administration of iodine Twenty four hours after last dose Fifteen days after last dose Twenty five days after last dose	0.160 0.090 0.240 0.160

The determination of iodine in the blood was then made during the administration of iodine as well as after. The same procedure was used as before, the dogs were on the same diet, and special care was taken in the amount of salts added to the food and the selection of the food. The results are summarized in Table IV.

In the interpretation of the above results it must be taken into consideration that the elimination of iodine is very rapid. From 65 to 80 per cent appears in the urine within twenty four hours after its administration.

TABLE IV

SPECIMEN	CONTENT OF IODINE	
	MG PER 100 CC	
	OF BLOOD (KENDALL'S METHOD)	
1 Dog 0.1 gm iodine daily for twenty days		
Analyses		
Before administration		0.146
Tenth day		0.152
Twentieth day		0.158
Ten days after discontinuance of administration		0.142
2 Dog Given 0.1 gm iodine and 0.5 gm phosphates daily for twenty days		
Analyses		
Before administration		0.128
Tenth day		0.181
Twentieth day		0.276
Ten days after discontinuance of administration		0.167

From Tables III and IV it appears that in the animals taking phosphates and iodine, there is a retardation of the elimination of iodine, and after the administration is discontinued it takes ten to twenty days for the return to the normal iodine content.

#### CONCLUSION

The retardation of the elimination of iodine, and the failure to cause experimental hyperthyroidism in animals taking iodine in conjunction with phosphates can be explained by the assumption that phosphates exert a depressing action upon the "endocrine and sympathetic systems" which control the function of basal metabolism.

The practical benefit derived from this combination of iodine and the phosphates is apparent, the danger of iodine is minimized, and at the same time the organism uses more iodine due to the slow elimination.

#### REFERENCES

- <sup>1</sup>Martinet, A. Les Medicaments Usuels, 1912, p. 76
- <sup>2</sup>Kendall and Richardson Jour Biol Chem, 1920, xli, 168
- <sup>3</sup>Von De Quervain Ruck und Ausblicke in der Schilddrusen Pathologie, Mitt u d Grenzgeb d Med u Chir, 1926, xxxix, 415

# LABORATORY METHODS

## METHODS OF TESTING ANTISEPTICS\*

BY GEORGE F. REDDISH, PH D, BALTIMORE, MD

FOR a great many years the bactericidal efficiency of antiseptics have been determined by what is known as the "phenol coefficient test." In this country two tests have been used for this purpose, namely, the Rideal Walker<sup>1</sup> and the Hygienic Laboratory methods. The first named test was published in England in 1903 as a method for determining the germicidal value of disinfectants as compared to phenol. Since in England antiseptics are understood to be substances which kill microorganisms,<sup>2</sup> they also were tested by the Rideal Walker method. It was consistent at that time to compare all germicides to phenol, since this was, and still is a widely known antiseptic of very constant properties. The result has been that all kinds of germicides have been compared to phenol and their relative value expressed as a 'phenol coefficient.' While this test has served its purpose admirably during these twenty and more years, it has all this time been misleading in one particular, that the ability to kill the test organism used, *B. typhosus*, does not necessarily mean that other pathogenic bacteria will also be killed by the germicides tested in this way. There is much information available now to show that such is not the case.

Disinfectants and antiseptics are used to kill pathogenic microorganisms and not simply for killing *B. typhosus*. Tilley<sup>4</sup> has stated that "a phenol coefficient has no practical value whatever, except as a means of preventing the use of positively worthless preparations and as an aid to the manufacturer in maintaining uniformity of his product." This is in keeping with the present trend of recognizing the "specificity" of germicidal action of disinfectants and antiseptics. I<sup>5, 6, 7</sup> have approached the subject in a somewhat more positive manner and have suggested definite organisms, representative of the main group of pathogens, to be used in a test for determining the bactericidal efficiency of germicides. According to this scheme phenol coefficients could still be used by the manufacturer for controlling the uniformity of his products but the dilutions of these products which are specified for actual use must be based on definite tests against the organisms it is desired to kill. Antiseptics for use on the skin and mucous membranes and in cuts, abrasions, wounds, etc. should be tested against pyogenic organisms such as *Staphylococcus aureus* and *Streptococcus hemolyticus*. If a disinfectant is recommended for general use, the manufacturer must be sure that the dilutions specified for his product will kill all of the non

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Those preparations which are sold in tablet or powder form from which antiseptic solutions are made by the addition of water are examined by the test outlined above

#### ANTISEPTIC OIL PREPARATIONS

Many antiseptic preparations have the active ingredient incorporated in some inert mineral oil. Regardless of the nature of the oil base which carries the active ingredient, the test as outlined above would not be applicable. Instead a method which we call the "Filter Paper Method" is used. This test is, briefly, as follows:

No. 2 Whatman Filter paper is cut into pieces about 0.5 cm. square, placed into a test tube, plugged with cotton and sterilized in the hot air oven at not over 170° C. (to prevent charring). The desired number of these sterile squares are then immersed in a twenty-four-hour broth culture of the test organism, *Staphylococcus aureus*. (This culture must be a fresh resistant strain which is not killed by a 1-70 phenol in ten minutes, nor by 1-80 phenol in fifteen minutes at 20° C., following the method given above.) These paper squares, impregnated with the culture of *Staphylococcus aureus*, are then fished out with a sterile culture wire (bent on the end) and transferred to a tube of broth (10 cc.) of the above composition. By shaking thoroughly at intervals over a period of five to ten minutes, the excess of the antiseptic oil is washed off from the paper squares. The pieces of paper are then fished out and transferred to another tube of broth (10 cc.) and incubated at 37° C. for forty-eight hours. The tubes are then observed for growth.

Some of the antiseptic oil preparations are recommended for use in such a way that only short time application is involved. If they are recommended for use in surgical dressing where contact with the infective organisms is assured over a long period of time, an inhibitory test would then be applicable. (See method for antiseptic dyes.)

#### POWDERS

Under this heading are included all powder preparations which claim to be antiseptic and which are applied directly to the skin, mucous membranes, and infected surfaces. Those powders which are used in such a way that no conditions are established whereby they are kept in continued contact with the infected surface, such as talcum powders, tooth powder, etc., are examined by the "Filter Paper Method" as follows:

Sterile filter paper squares impregnated with *Staphylococcus aureus* as outlined above, are covered on both sides with the powder and allowed to remain in this intimate contact for five minutes. At the end of this time period the piece of paper is fished out and transferred to a tube of broth and, after the adhering powder is shaken off, again transferred to broth and incubated at 37° C. for forty-eight hours. These powders which are recommended for dusting into infected wounds may be examined as outlined later.

## ANTISEPTIC TOOTH PASTES AND SHAVING CREAM

In the examination of these two types of preparations, we are faced with the necessity of determining their efficiency in the undiluted state because it is practically impossible to fix a definite dilution of each product which will simulate the dilution of the preparation when used in practice. We have, therefore, seen fit to test the antiseptic properties of these preparations undiluted. Contact with infective organisms is very short at best, and a test to determine killing effect is applicable. The "Filter Paper Test" is used for this purpose and the procedures as outlined above are followed, *Staphylococcus aureus* being the test organism. In this case the impregnated pieces of paper are completely covered with the paste and this contact allowed to continue for five minutes at 37° C.

Because of the fact that some dentifrices may be more effective in solution than in the paste form, a 50 per cent solution is also made and tested by the filter paper method in the same manner as indicated for the undiluted paste. The active ingredients have a better opportunity of coming into contact with the bacterial cells when in a solution. If the tooth paste or shaving cream kills *Staphylococcus aureus* either in the undiluted form or in a 50 per cent solution (1—1) in five minutes at 37° C, it will be considered as an antiseptic preparation.

## ANTISEPTIC LOZENGES AND ANTISEPTIC SUPPOSITORIES

Lozenges and suppositories for which antiseptic claims are made are examined in a manner which approximates to a great extent the conditions met with in practice. These preparations dissolve slowly giving a solution of their active ingredients in the local secretions. There is no way of accurately determining the concentration of solution obtained in the practical use of such products. For this reason we simply test these preparations in a concentrated aqueous solution. In this case more benefit is given the product than is probably justified, but considering all the factors involved this is the fairest method of testing. The filter paper method is then used as follows: pieces of paper impregnated with *Staphylococcus aureus* are allowed to remain in a saturated aqueous solution of the product for as long a time as is required for the preparation to dissolve when used as directed. For the antiseptic lozenges this requires about ten to fifteen minutes. Some suppositories require a longer time and the period of the test is regulated by the time it takes for solution in distilled water. The usual procedure for the filter paper test is followed.

## ANTISEPTIC SOAPS

For the testing of antiseptic soaps the filter paper method is applicable. In fact filter paper simulates fairly closely the conditions found in the skin. In the case of liquid soaps, the undiluted soap is used in the filter paper test as outlined above. If the soap is in powder or cake form, a thick lather is made aseptically in sterile water in a sterile Petri dish, and the filter paper test carried out in the usual manner.



# ANTISEPTIC SALVES, OINTMENTS, BUNION PADS, CORN PLASTERS, SURGICAL DRESSINGS AND POWDERS FOR WOUND DRESSINGS

When such preparations as these are used in practice, the active ingredients are held in intimate contact with the infective microorganisms and can render them innocuous by simply preventing their activity. Since these preparations do remain in contact with the infected surfaces for long periods of time when used in practice, laboratory tests for their efficiency should simulate these conditions as nearly as possible. It is evident that such tests as have been outlined above would not be applicable, we are not interested here in killing organisms within a certain length of time. If an antiseptic salve, for example, does prevent the growth of microorganisms in an infected area, it will not only prevent them from doing harm, but will also render them easy prey for the leucocytes. For this reason, the following method is applicable for this type of product.

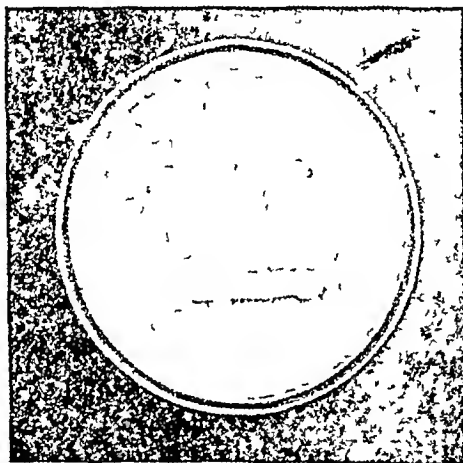


Fig. 1.—Illustrating the activity of an antiseptic ointment against *Staphylococcus aureus*. The dark streak is antiseptic ointment which is surrounded by a clear zone in which no organisms have grown. The white streak is control ointment without antiseptic. The surrounding turbidity is a heavy growth of *Staphylococcus aureus*.

*Staphylococcus aureus* of normal resistance<sup>5</sup> is grown at 37° C in the broth described above and transferred in this medium for three consecutive days. One-tenth of a c.c. of a 1-100 dilution of this culture is added to 15 c.c. of melted 10 per cent serum agar (15 c.c. sterile normal horse serum added to 15 c.c. of nutrient agar [15 per cent] made from the above broth base) at 45° C, the culture thoroughly mixed in the serum-agar and poured into a sterile Petri dish and allowed to cool at room temperature. As soon as this inoculated agar has hardened, the salves and ointments, previously melted at 37° C, are streaked over a small surface of the inoculated agar with a sterile glass rod. Melted sterile vaseline is spread on another part of the inoculated agar in the same manner and the plate, inverted, incubated at 37° C for forty-eight hours. After being incubated it will be noted that colonies of *Staphylococcus aureus* grow immediately adjacent to the vaseline

<sup>5</sup>In all of these illustrations mercurochrome was used to illustrate the methods discussed.

control and even under it. There is no active ingredient in pure vaseline which will prevent the growth of *Staphylococcus aureus*. However, in effective antiseptic salves and ointments a part of the active ingredients contained in them is absorbed into the serum agar, which is about one eighth of an inch deep, and by their presence prevent the organism present from growing (see Fig 1)

The plate will show a clear zone around the antiseptic salve or ointment which is in marked contrast to the turbidity of the surrounding medium caused by the heavy growth of the organism. In treating infected surfaces with preparations of this nature, it is necessary that the active ingredients leave the inert base and become free to surround the infective organisms. It is only in this way that the preparation will be of benefit in preventing the growth of or killing these microorganisms. If the antiseptic were so

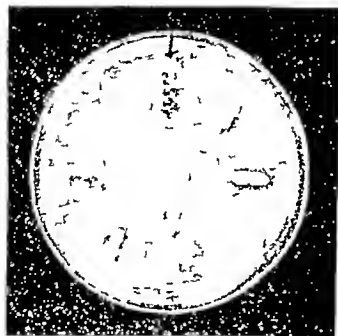


Fig. —This is a comparison of eight so called antiseptic ointments obtained from the retail market. Five of these have absolutely no effect on *Staphylococcus aureus*, one slight effect and two show a definite zone of sterilization.

securely incorporated in the inactive base that it could not become free to attack the microorganisms or if the base were of such a nature that the antiseptic could not separate from it, the value of such preparations so far as the antiseptic ingredient is concerned would be lost. Serum agar simulates fairly closely the conditions met with in skin and wounds\*. It is permeable, semisolid, isotonic, contains a body fluid, and constitutes a laboratory means of approximating the conditions found in human and animal tissues at least so far as the preparations under consideration are concerned. The clear zone may indicate germicidal action and not just bacteriostatic effect. To prove this, small pieces of agar from various parts of this zone are inoculated into broth and incubated for forty-eight hours. When the active ingredient comes out in sufficient concentration to be germicidal in the clear zone this is of even more interest because it indicates greater practical value of the preparation.

\*The addition of normal serum to 1 per cent nutrient agar gives a medium which closely simulates practical conditions.

As a matter of interest a number of widely advertised so-called antiseptic ointments which at this time enjoy a very wide sale were tested on a *Staphylococcus aureus* poured plate (see Fig 2) It will be noted that six of these ointments have no effect whatever against *staphylococcus aureus* while two of them give a definite zone of sterilization Antiseptic ointments should certainly kill or inhibit *Staphylococcus aureus* In this test if the active ingredients will leave the ointment and go into the surrounding medium they can inhibit *Staphylococcus aureus* and cause a clear zone, but these six ointments do not even do this It can be safely said that they will not be effective when used in practice

In testing bunion pads, corn plasters, adhesive, surgical dressings, and powders for wound dressings for which antiseptic claims are made, the procedure just given is employed These preparations are simply placed on top of a poured serum-agar plate containing *Staphylococcus aureus*, and incubated

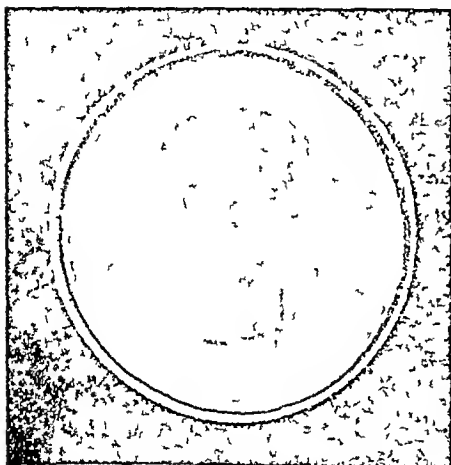


Fig 3—This illustrates the bactericidal efficiency of an antiseptic gauze as compared to gauze which is supposed to contain an antiseptic The wide zone around the upper antiseptic gauze shows that the active ingredient has passed out into the surrounding medium

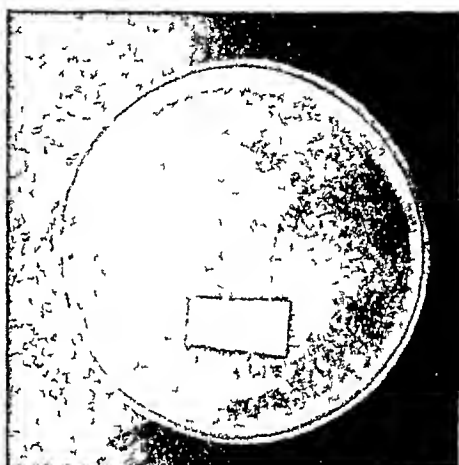


Fig 4—This shows an antiseptic adhesive as compared with ordinary adhesive the dark strip above being that which contains the antiseptic These are placed on the surface of the medium

at 37° C for forty eight hours The antiseptic in such products permeates the serum-agar medium and prevents the growth of the test organism (or actual killing may take place) The amount of antiseptic contained and its solubility, together with the ease with which it leaves the base, are factors in determining the width of the clear zone around these preparations (see Figs 3 and 4)

This same serum-agar plate method can be used for testing antiseptic catgut In this case pieces of the catgut are imbedded into the shake serum-agar culture of *Staphylococcus aureus* after it has been poured into a plate and cooled, it is then incubated at 37° C (see Fig 5) Fig 6 illustrates the relative merits of five different antiseptic catguts obtained from the retail market It is to be noted that number three is without any effect on *Staphylococcus aureus*, whereas Nos 1 and 4 are of slight effect, while Nos 2 and 5 give a wide zone This is about the only way this type of preparation can be tested

## ANTISEPTIC DYES

In examining antiseptic dye solutions we must consider that we are dealing with a liquid antiseptic whose action is supposed to continue for a long time. When these dyes are applied to infected surfaces or wounds they are expected to penetrate tissue and continue their activity. Because these dyes remain

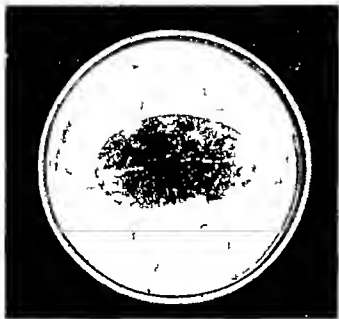


Fig 5—In this case antiseptic catgut is imbedded in the agar. Penetration of 16 cm is shown as well as to a depth of one-eighth of an inch.

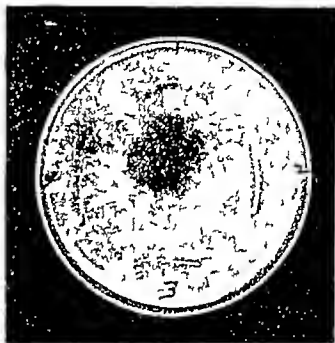


Fig 6—Comparing the efficiency of five different antiseptic catguts obtained from the retail market. No 3 has no bactericidal effect against *Staphylococcus aureus*. Nos 1 and 4 slight effect whereas Nos 2 and 5 have considerable penetration and bactericidal action.

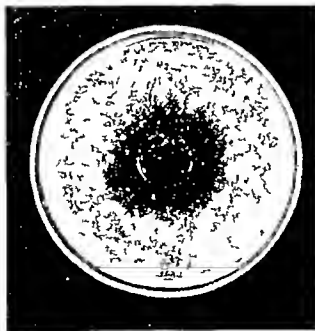


Fig —This illustrates the penetration and antiseptic activity of a liquid antiseptic dye. This antiseptic penetrates the medium for 1 cm from the edge of the cup.

for an indefinite time in the tissue to which they are applied the test which simulates this condition must also be used. For this purpose the serum agar Cup Plate Method is applicable\* (see Fig 7). The proper amount of staphylo

I am indebted to Dr L. C. Himebaugh of the Pease Laboratory of New York City for this method.

coccus aureus culture is added to 15 cc melted serum-agar cooled to 45° C (as described above) and poured into a sterile Petri dish. A sterilized glass stopper 1.5 cm in diameter is then placed in the center of the dish and left until the agar has hardened. The glass stopper is then removed by twisting slightly and the small cracks and crevices are sealed with about 3 drops of sterile agar. The antiseptic dye solution to be tested is placed (0.25 cc) into this cup-like depression and the plate covered with an unglazed clay top. This is then incubated at 37° for twenty-four to seventy-two hours. If the antiseptic penetrates the medium in germicidal or bacteriostatic strength there will be a clear zone around the cup (see Fig 7). Subcultures into broth from different parts of the zone are necessary to determine whether or not the substance is germicidal or merely bacteriostatic. This method is of value also in testing the ability of antiseptics to retain their strength over a long period of time. One of the common antiseptics<sup>\*</sup> in use today was so tested after five years in solution and gave a zone equal to that formed by a fresh solution. It is also useful for testing the penetrating power of any liquid antiseptic.

#### SUMMARY

Methods for testing the efficiency of various kinds of antiseptic preparations are briefly given. Laboratory tests cannot, of course, simulate the exact conditions found in practice, but the procedures outlined here are an attempt to do this and apparently they do approximate these conditions to a very marked degree. More complicated methods would be expensive, involved and time-consuming, and would probably introduce factors which would make the tests more difficult to duplicate. If substances prove to be antiseptic by the tests outlined here, there is every reason to believe they will be efficacious when used in practice.

#### REFERENCES

- 1 Rideal, S., and Walker, J. T. A. Examination of Disinfectants, J. San. Inst. 24 424 441, 1903, Approved Technique of the Rideal Walker Test, London, 1921
- 2 Hygiene Laboratory Bulletin, No. 82, April, 1912
- 3 Muir, R., and Ritchie, J. Manual of Bacteriology, London, 1919, p. 166
- 4 Tilley, F. W. Phenol Coefficients, Am. J. Pub. Health 11 513 519, 1921
- 5 Reddish, George F. The Resistance to Phenol of Staphylococcus Aureus, Am. J. Pub. Health 15 534 538, 1925
- 6 Reddish, George F. Determining the Germicidal Efficiency of Disinfectants, Am. J. Pub. Health 16 283 286, 1926
- 7 Reddish, George F. Examination of Disinfectants, Am. J. Pub. Health, 17 320 329, 1927
- 8 Reddish, George F. Methods of Testing Antiseptics, Drug Markets 20 495, 1927
- 9 Editorial, J. Am. Pharm. A. 16 501 2, June, 1927

DEPARTMENT OF BACTERIOLOGY,  
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<sup>\*</sup>One per cent solution of mercurochrome

## A SOURCE OF INCONSTANT ERROR IN THE DARE HEMOGLOBINOMETER\*

BY FRANK G. HAUGHWOUT, MANILA, P. I.

CLINICAL pathologists, physicians, and others to whom falls the task of making routine hemoglobin estimations, have long realized the inaccuracies inherent to the so called "rapid clinical methods." The Newcomer disc method and the procedures of Colin and Smith, and of Palmer, all yield results of far greater accuracy than the apparatus of Tallqvist, Sahli, Dare and others, but for the most part they are impracticable in the office of the average physician, and are so considered by many laboratories of some pretension.

The instrument introduced in 1900 by Dare<sup>1</sup> is based on sound principles and is so eminently practical in its construction and mode of use that its adoption has become widespread until it now is looked upon as the most accurate of the instruments for rapid hemoglobin determination. It, accordingly, has been adopted by a considerable number of large institutions. However the instrument has defects which, to me, seem to be due more to carelessness in its manufacture than to inherent errors in principle.

Sentry in a comparative study of different methods of hemoglobin determination has shown that the instrument reads too low in the upper part of the scale, that is to say, above 65 per cent. He also has shown that these errors are, to a large degree, modified by grinding off 15 per cent of the upper end of the scale as suggested by Sanford.

I discovered another source of error lying in the pipettes into which the blood is drawn. As I have seen no mention of this fallacy in the current literature, I am putting it out as a warning to other workers who may not be aware of the possibilities for error in their own instruments. The discovery came about in this way:

An order had been placed through a local importing house for a Dare hemoglobinometer. Two extra pipettes also were ordered to provide against the contingency of breakage. The two extra pipettes were delivered several weeks before the instrument and its accompanying pipette. The three pipettes were placed in service to be used interchangeably with the instrument it being assumed that they were standardized. Unfortunately, it is not possible to say which of them was delivered with the instrument. It is not essential to the purposes of this discussion whether any one of them was accurate. The main point is that no two of them agreed within even 8 per cent. Prior to the delivery of the Dare instrument hemoglobin estimations had been made with the Tallqvist scale, the standard directions for its use being closely followed. Several weeks before the delivery of the Dare instrument I had, at the

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instance of Dr H E Stafford, of Manila, undertaken the study of the bowel discharges of a local business man In the course of the investigation it seemed desirable to examine the blood, so erythrocyte counts and hemoglobin determinations were made at intervals, the latter being made with the Tallqvist scale The hemoglobin findings were as follows

January 27-----	75 per cent
March 3-----	70 per cent
March 26-----	75 per cent

At this point the patient went up into the hills where he remained until April 17 A few days later he came to the laboratory It was found that his erythrocyte count (taken with a Trenner pipette each time) had risen from 4,670,000 to 5,220,000 The Dare instrument having arrived, his hemoglobin was taken by it and was found to be 64 per cent This was startling, but the colors appeared to match perfectly so the matter was reported to Dr Stafford A few days later the patient went to another laboratory for a Wassermann test There he spoke of the apparent fall in his hemoglobin whereupon the physician there examined his blood with another Dare instrument and told the patient that his hemoglobin was "nearer 90 per cent than 60 per cent" The patient naturally reported this to his physician who in turn communicated it to me

On learning this I communicated with the physician who had made the second determination and suggested the desirability of a comparison of the two instruments He, on his part, contended that the instruments were standardized at the factory and it was unlikely that a discrepancy could exist between them His lack of interest in the matter led to the dropping of that part of the investigation

At my request, Dr Stafford sent the patient to the laboratory on April 23 His hemoglobin was read again by the Dare instrument, both by me and by his associate, Mr John K Pickering, of the biologic staff of the Pathologic Society I made a reading of 76 per cent while Mr Pickering read 73 per cent In each of these instances (April 17 and 23), pipettes were taken from their boxes at random, so it is impossible to say which of the three was used in either instance The obvious discrepancies in these two readings seemed to indicate the necessity for an inquiry into the reasons thereof

Mr Pickering kindly volunteering to furnish the blood and to assist in a series of experiments, an investigation was started without, however, any definite idea as to just where the trouble lay A number of readings were taken on different days, the pipettes being taken at random, with the result that conflicting and irreconcilable percentages were obtained On going over the figures, however, a marked grouping was noted and ultimately it was found possible to arrange the readings in two groups which, for convenience, may be designated as the 58 to 60 per cent and the 70 per cent groups This gave a clue to the situation

The pipettes were separated and designated A, B, and C respectively, and readings made by them were so classified Each pipette was read four times, twice by Mr Pickering and twice by myself, on each of four separate days, each observer accordingly, having read each pipette eight times In every

case the blood was taken from Mr Pickering. The readings were consistent throughout, showing only the minor variations customarily observed. All readings were by candle light in a closet. The results are summarized in the averages shown in Table I.

TABLE I

PIPETTE	PER CENT HEMOGLOBIN	
	HAUGHWOUT	PICKERING
A	75½	73½
B	58¾	58¾
C	82½	82½

This made it apparent that there was a constant difference of more than 20 per cent between the readings with Pipettes B and C on identical blood. It seemed obvious that the capillary chambers must be of unequal depths.

To ascertain if this were so, measurements were made with the ocular micrometer on a compound microscope of the distance separating the two glass elements forming the capillary chamber, that is to say, the depth of the

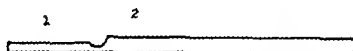


Fig. 1—Points of measurement of capillary pipette

chamber. The glass plates were clamped in the regular holder and the objective was focused down on the inner edges and the distance separating them measured. The measurements obtained are shown in Table II.

TABLE II

PIPETTE	DEPTH MILLIMETERS
A	0.175
B	0.140
C	0.192

These measurements are seen to be consistent if one compares them with the hemoglobin readings given in Table I. However, the beveling of the glass made correct focusing rather difficult, thus creating a possible source of error, so it was decided to repeat the measurements using a micrometer caliper. Unfortunately one reading in the metric system could not be obtained but a Starrett instrument measuring in thousandths of an inch was kindly loaned by the Atlantic Gulf and Pacific Company of Manila. The white glass element of each pipette was measured at the points indicated in the diagram composing Fig. 1.

The values obtained by these measurements are set forth in Table III.

TABLE III

PIPETTE	1	2	DEPTH OF CHAMBER
A	91.5	98.5	7.0
B	86.0	92.5	6.5
C	87.0	92.5	7.5



While no claim is made for absolute accuracy in these measurements it will be seen that they run parallel with the hemoglobin readings

To test the matter, eight readings (four by each observer) were made on the same day with Pipette B. The first four were made with the pipette as it stood, and an average reading of  $59\frac{1}{4}$  per cent was obtained. Then, a slip of paper measuring 0.003 inch in thickness was clamped between the two glasses and the pipette again filled. This time it gave readings averaging 92 per cent

#### DISCUSSION

Dare lays stress on accuracy in the depth of the capillary chamber although he does not say what this should be. He states that the depressed surface of the white glass is ground and "measured with a micrometer to give an exact depth." He adds that when clamped, the chamber "contains a blood-film of definite thickness," and finally, "The pipettes all contain a blood stratum of standard thickness, and can therefore be used with any instrument, a distinct advantage over the calibrated pipettes which have a capacity only adapted to one particular apparatus."

It is very evident that the pipettes that form the subject of this discussion do not conform to these specifications, and it is an entirely legitimate subject for disquieting speculation as to just how many similarly inaccurate pipettes are distributed about in various laboratories and in daily use. So long as one pipette is continuously used with a given instrument and substitution by another is made only when the first is broken, the readings will be relatively consistent, be they accurate or inaccurate. Unless the same group of patients is under study, the eventual substitution of another pipette that may be read either higher or lower than the first may not be followed by a series of readings that of themselves would lead to lurking doubts in the mind of the user. Unless some one else has occasion to check his reading on the blood of some particular person, as happened in the instance recorded in this paper, the hematologist may continue to use an inaccurate pipette indefinitely, serenely unconscious that his determinations are inaccurate, or that the pipette he is using today may differ in its readings by 10 or even 20 per cent from the one he used a few months ago.

The three pipettes composing the set in my possession presented a definitely graded set of readings. Selected at random they might either show a progressive improvement or a progressive deterioration in the condition of the blood. On the other hand, they might yield a set of irrational and widely divergent readings that in the end would most assuredly destroy the faith of the clinical man in his hematologist.

#### CONCLUSIONS

1. Senty already has indicated the fallacies that lie in the upper segments of the comparison prism of the Dare hemoglobinometer. These errors are constant and may be largely compensated by grinding off a portion of the upper segment of the prism.

\*Senty says it is about 0.18 mm.

2 It has been shown in this paper that inaccurate grinding of the capillary chambers of the blood pipettes leads to the production of chambers of unequal depths that accordingly yield inaccurate values with the blood sample. These errors, of course, are inconstant and their extent cannot be known to the user of the instrument. Grave errors in hemoglobin determination naturally follow and these cannot fail to exert a profound influence on treatment and prognosis, to say nothing of diagnosis.

3 Both sources of error may be avoided by greater attention on the part of the manufacturers to the original specifications for the construction of the instrument as set forth by Dare in his original paper.

4 All Dare instruments at present in use should be checked as to color prism. More important, however, than this is the standardization of every pipette with a view to discover if the chamber is of standard depth to harmonize with the values expressed by the comparison prism.

#### REFERENCES

- 1 Dare, Arthur. A New Hemoglobinometer for the Examination of Undiluted Blood. *Philadelphia Med J* 6: 557, 1900.
- 2 Senty, Elmer G. A Comparative Study of Various Methods of Hemoglobin Determination, *J LAB & CLIN MED* 8: 591, 1922-23.

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## A TECHNIC FOR STAINING RED BLOOD CELLS FOR MEASUREMENT OF CELL DIAMETERS BY THE PROJECTION METHOD\*

BY FRANCIS P. PARKER, B.S., EMORY UNIVERSITY, GA.

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**FIX** smear by ordinary heat method. Apply a saturated solution of eosin in 95 per cent alcohol for two minutes and wash with distilled water and drain. Next, flood with a 1 per cent water solution of methyl violet. This is left on for two minutes, washed with distilled water and the slide dried over a flame.

The above procedure stains the red cells a deep bluish violet, and the white cells a light violet with deep nuclei. In the measurement of red cell diameters it is necessary to have sharp contrast between the cell margin and background and this stain supplies this feature, accentuating the cell outline much more than Wright's or other stains common in blood examination.

It is essential that the order given above be carried out due to the action of alcohol in preparing the cell for the reception of the water soluble methyl violet. Otherwise best results are not obtained. The heat fixation method proved preferable in simplicity and shorter staining time to both the methyl alcohol and absolute alcohol methods.

LABORATORY OF BIOLOGICAL CHEMISTRY

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# MICROSCOPIC PROJECTIONS IN THE MEASUREMENT OF ERYTHROCYTES\*

BY FRANCIS P. PARKER, B.S., GEORGE T. LEWIS, PH.D., AND  
J. L. MCGHEE, PH.D., EMORY UNIVERSITY, GA.

THE use of the microscope as a projection apparatus has long been in practice in embryologic work where no special attention was given to the exact magnification of the projected image. To achieve this an ordinary monocular microscope of the type generally used by medical students is employed. It is broken at the inclination point so that the long axis of the body tube is at right angles to the pillar. The draw-tube and collar in which it slides is removed from the body-tube. The upper planoconvex lens of the Abbe condenser and the mirror are also removed. This arrangement permits the scope to be fixed in a position with both the base and the tube perpendicular to the horizontal, and the source of illumination immediately above the condenser. Where large magnification is desired, the intensity of illumination must be great. In this application a 250 watt flood light with concave reflector and planoconvex lens, three inches in diameter, was used. Both the scope and light box are fixed with set screws on a metal bar set upright in a 20 inch by 20 inch, two inch oak base, heavy enough to act as an anchor for the apparatus. The image is projected on a sheet of white paper thumb-tacked to the base.

In using this apparatus for measuring blood cell diameters, the distance between the surface of the stage and the surface of the base must be exactly 36 inches. When the Leitz  $\frac{1}{12}$  inch, 18 mm., objective, oil-immersion lens is used, this distance gives a magnification of 500 diameters. Therefore, the projected red cell is measured in two directions in millimeters, and the sum of the two measurements is the red cell diameter in microns.

With this method it is possible to take cell measurements to 0.25 microns. The conditions governing the selection of cells to be measured and the number of cell measurements required for a diagnosis are the same as those already set forth.

The magnification may be conveniently checked from time to time by use of the standard Levy counting chamber. The ruled squares are  $\frac{1}{400}$  mm. in area so when projected at 500 diameters they should measure 25 mm. across. The chamber is reversed to discount error due to thickness of the plate.

## ADDENDUM

Since the above was written, we have adopted a 400 watt flood light bulb as the source of illumination. This gives a brighter field and less eye strain when a series of determinations are to be made. We have also attempted to increase the magnification by means of a projection ocular, but the amount of light is cut down to such an extent as to prohibit its use.

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# THE HINTON GLYCEROL CHOLESTEROL PRECIPITATION TEST FOR SYPHILIS

A COMPARATIVE STUDY WITH THE WASSERMANN TEST IN 2120 CASES\*

By EDGAR J. MUNTER, M.D. BOSTON

THE Wassermann reaction long has been the standard serum test for syphilis, in spite of the fact that it is not infallible and in spite of its technical difficulties. Many new tests for syphilis of greater simplicity have been described, but have not proved to have greater delicacy or accuracy. In June, 1927, Hinton described a new, simple easily read sensitive test for syphilis which required less labor, apparatus, and material than the Wassermann reaction, which had fewer false positives and which was more sensitive than either the Wassermann or Kahn reaction. This report consists of an analysis of 2331 consecutive Wassermann and Hinton glycerol cholesterol reactions on 2120 patients in the Peter Bent Brigham Hospital and its out patient department between the months of November, 1927 and April, 1928.

The laboratory tests upon which this paper is based were performed as follows:

## 1 *Wassermann Reaction*—The technique is that published by Hinton.<sup>1</sup>

Two antigens were used separately, one a simple, cholesterolized alcoholic extract of guinea pig heart, and the other an alcoholic extract of human heart half saturated with cholesterol at 17° C. The hemolytic system consisted of 2 units of amboceptor, 2 units of complement and 0.5 cc. of a 5 per cent suspension of sheep's corpuscles. One tenth cubic centimeter of serum was used for each antigen, and 0.2 of a cc. for the auticomplementary control. The primary incubation was for forty minutes at 37° C. and the secondary incubation for one hour at the same temperature. Tests were read immediately after the secondary incubation. They were reported as positive, negative, and doubtful.

## 2 *Glycerol-Cholesterol Precipitation Reaction*—The glycerol cholesterol precipitation reaction, which Hinton believes is, in reality, not a precipitation test, but an agglutination reaction, was executed as described in his article in the *Boston Medical and Surgical Journal*<sup>2</sup> with the following modifications which are soon to be published in detail by Hinton.

(a) His stock indicator consisted of the ether insoluble alcohol soluble fraction of beefsteak muscle which he added to a 0.7 per cent solution of cholesterol in absolute alcohol, in the proportion of one part of the extract to nine parts of the cholesterol solution. (b) The temperature of incubation was 37° C. rather than 27° C. (c) The amounts of serum used were 0.1, 0.2, 0.3, 0.4, and 0.5 of a cc. (d) The salt concentration varied in proportion to the amount of serum used, as given below.

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latent cases and legitimate misses for both tests, for these 11 were all early cases of syphilis which had been treated intensively and had been serologically negative for some time. This leaves us with the conclusion that the Wassermann test was positive in 72 per cent of the cases of clinical syphilis (treated and untreated), while the Hinton test was positive in 89.7 per cent, and that there were 26 cases of syphilis which the Wassermann would miss entirely, but only 10 which would have been missed by the Hinton test.

The Wassermann reaction was doubtful 30 times in the presence of syphilis and 25 times in nonsyphilitics, whereas the Hinton test was doubtful in 11 syphilitics and in 25 nonsyphilitics. In this series a doubtful Wassermann had just about an even chance of occurring with syphilis, while a doubtful Hinton test two out of three times occurred in nonluetics.

TABLE II

	WASS +	HINTON +	WASS -	HINTON -	WASS ±	HINTON ±
Cases of Syphilis	148	184	37	21	30	11
Nonsyphilitic Cases	11	7	1847	1851	25	25
Doubtful Cases	1	18	14	2	7	2

Both the Wassermann and Hinton tests were positive in 145 cases. One hundred and forty of this group were cases of proved syphilis, and the remaining 5 were cases who clinically were suspected of syphilis, but who have not returned for further study. In every case in which a positive Wassermann was accompanied by a positive Hinton test, syphilis has been present, and this information alone would make the Hinton test invaluable as a supplementary test for syphilis.

Both reactions were negative in 1825 cases. Eighteen hundred and twelve of these, or 99 per cent, were in patients who had no history or clinical evidence of syphilis. Two cases of central nervous system syphilis under intensive treatment gave negative blood reactions, as did the 11 cases referred to above as cured or latent cases of syphilis. In only 2 cases out of 1825 did the combined negative result occur with active syphilis, another fact which makes the confirmatory evidence of the Hinton test valuable.

There were 13 cases in which the Wassermann reaction was strongly positive and the Hinton test negative. Eight of the 13 were obviously false positive Wassermans and have had subsequent negative reactions. Of the remaining members of this group two were young married women without a single sign or symptom of syphilis, whose husbands were nonsyphilitic clinically and serologically and whose children were not luetic. We are inclined to consider these women as nonluetie in spite of the fact that each has had three doubtful Wassermans and two positive Wassermans though they are being further studied to determine their true status. One other case gave a history of a questionable sore, but is not available for further study. Another case was one of active meningo-vascular syphilis and was definitely a miss for the Hinton test. A young, pregnant, single girl falls into this group with three doubtful Wassermans and one positive Wassermann, but we do not as yet feel certain as to the presence or absence of syphilis in this individual. The outstanding fact in this group is that, of the 13 cases with positive Wassermans and negative

Hinton tests, eight, and probably ten, cases were mistakes in the Wassermann, whereas the Hinton test was definitely wrong only once and questionably in two instances

There were 41 cases where the Wassermann was negative and the Hinton positive. Twenty of these were definitely syphilis which the Wassermann missed, and 16 of these 20 were instances of the occasional negative and occasional positive which are often obtained during the course of late treated syphilis, cases apparently not cured of their syphilis. One other case gave a history of a hard chancre with local treatment only, and was missed entirely by the Wassermann. Another instance was that of a 56 year old woman with a saddle nose, saber tibiae, interstitial keratitis, and a history of snuffles and a rash as an infant, an untreated congenital luetic. Another case had a gumma clinically, which responded promptly to antiluetic treatment and, though the Wassermann has never been positive, the Hinton test was positive. An old treated case of *tabes dorsalis* had also repeatedly negative Wassermanns and positive Hinton tests. There were seven cases in this group with falsely positive Hinton tests, as proved by autopsy or further clinical and serologic study. The remaining 14 have not been available for further study. It is of interest that 12 of these 14 are over forty-five years of age and suggests that if they could be followed they might be found to be old, "burned out" cases which the Wassermann misses, for many of them have symptoms which would be explained by syphilis.

There were 23 cases with doubtful Wassermann tests and positive Hinton tests and all were cases of proved syphilis, except 4 cases of doubtful diagnosis. On the other hand, there were 36 cases with doubtful Wassermann tests but with negative Hinton tests, and 28 of these were not luetic. Five cases with this latter combination were old treated luetics, one was an untreated asymptomatic syphilitic and one other had *tabes dorsalis*. The positive or negative Hinton test, then, has been a quite accurate indication of the significance of a doubtful Wassermann.

The Hinton test was doubtful in 2 syphilitics with positive Wassermanns and in twenty-four nonluetics and four luetics with negative Wassermanns. Four treated cases of syphilis had both tests return doubtful.

Studying all these groups, we found that the Wassermann was correct in 1995 cases and wrong in 48, while the Hinton test was correct in 2034 cases and wrong in 20. This gives an error of 2.3 per cent for the Wassermann, as compared to 1.3 per cent error for the Hinton test.

Taking the cases with positive Hinton tests as a group, we found that we had 191 cases of known diagnosis and 18 doubtful cases. Of the known cases which had a positive Hinton test, 96.4 per cent had syphilis and 3.6 per cent were nonsyphilitic. We can only speculate upon which way the 18 doubtful cases would swing these percentages, but, from the large number of times that a positive Hinton test in a doubtful case meant a history of syphilis upon further study, it is our impression that these figures would not be greatly altered. On comparison with the Wassermann, we found that only 93 per cent of 160 known cases in which a positive Wassermann occurred were syphilitic and the rest nonsyphilitic.

There were 1874 cases with negative Hinton tests, of which 98.6 per cent were nonluetic, 0.7 per cent were cured or latent cases, and 0.4 per cent were cases of syphilis under active treatment. Only one case was an active, untreated meningo-vascular syphilitic. Of 1880 cases in which the Wassermann was negative, 98.5 per cent were nonluetic, 0.7 per cent were cured or latent, and 0.7 per cent were cases of syphilis under treatment. There were 3 untreated cases with clinical evidence of syphilis and negative Wassermans. The two tests differ here only in having more cases of treated late syphilis become Wassermann negative than become Hinton negative.

This brings up the point whether the persistently positive Hinton test in actively treated late syphilis is a real indication of the uncured condition of a

TABLE III

		WASSERMANN	HINTON	INTERVALS BETWEEN TESTS
A B	Nonsyphilitic Acute Prostatitis	Negative	Negative	
		Positive	"	21 days
		Negative	"	3 "
		"	"	4 "
E H K	Nonsyphilitic Acute Upper Respiratory Infection	"	"	7 "
		Positive	Negative	
		Negative	"	4 days
		"	"	21 "
E L	Nonsyphilitic Catarrhal Jaundice	"	"	4 "
		Positive	Negative	30 "
		Negative	"	
		"	"	30 days
H R	Nonsyphilitic Asthma	Positive	Negative	
		"	"	4 days
		"	"	10 "
		Negative	"	30 "
		"	"	4 "
		"	"	14 "
		"	"	10 "
		"	"	30 "
C S	Treated Syphilis	Negative Doubtful	Positive	30 days
H L	C.N.S. Syphilis	Negative Doubtful	Positive	4 days
		"	"	4 "
H S	Pregnancy	Doubtful	Negative	
		"	"	4 days
		Positive	"	7 "
		Doubtful	"	3 "
L L	Nonsyphilitic	"	"	30 "
		Doubtful	Negative	
		"	"	21 days
		Negative	"	14 "
H	Syphilitic Glossitis	"	"	7 "
		Doubtful	Positive	7 days
H C	Syphilis	Positive	Positive	
		Doubtful	"	3 days
		Positive	"	7 "
DeN	Nonsyphilitic Asthma	Doubtful	Negative	
		Negative	"	14 days
		Doubtful	"	30 "
		Negative	"	7 "
L J	Treated Syphilis	Negative Doubtful	Positive	90 "



patient whose Wassermann has become negative, or whether the Hinton test is due to a condition of the blood which occurs in an individual who has or has had syphilis. The fact that the actively treated primary and secondary cases, in which we feel more confident of cure, develop a negative Hinton as readily as a negative Wassermann is against the latter assumption. Time alone can tell us the true significance in the late cases, but the fact that those cases of late syphilis which we have seen develop negative Hinton tests have nearly always been those in whom cure is more likely, and the fact that a few cases in which the Wassermann has been negative and the Hinton positive have later developed signs of activity, make us feel that the Hinton test may prove to be a more accurate estimation than the Wassermann of the cure or arrest of syphilis.

A striking feature of the glycerol-cholesterol test is its consistency. It is well known that the Wassermann test will vary in its degree of positivity from time to time without any treatment at all. Our experience with the Hinton test shows that, with only six exceptions in 2331 tests, the reading once established remained constant. That is, the positive cases remained positive, unless influenced by long, intensive treatment, and the negative cases were persistently negative. Table III gives an idea of the marked difference in consistency of the tests.

In the seven cases of false positive Hinton tests, we found no definite association with acute infection or with any disease, except for the fact that 2 of the 7 cases were instances of cerebral vascular accidents and 2 were cases of diabetes. There was, however, among the erroneously positive and weakly positive Wassermann reactions a large number with acute respiratory infection. Thus, we found in this group three asthmatics during their acute exacerbations, one pneumonia, three acute upper respiratory infections, one lung abscess, one bronchiectasis, and one pulmonary tuberculosis. Only one false positive Wassermann was obtained with jaundice and two with diabetes. The remainder of the false positive and doubtful reactions were in miscellaneous conditions.

#### SUMMARY

1 There were 2331 consecutive Wassermann and Hinton glycerol-cholesterol precipitation reactions performed on 2120 patients.

2 Both tests agreed in 93.4 per cent of the cases, and in the 6.6 per cent where they disagreed the Hinton test was the one which agreed with the clinical evidence two times out of three.

3 The Wassermann was positive in 72 per cent of the cases of treated and untreated clinical syphilis, while the Hinton was positive in 89.7 per cent of this group.

4 In all cases where both tests were positive, the clinical diagnosis was syphilis, and in 99 per cent of the cases where both were negative, there was no evidence of syphilis.

5 Eleven false positive Wassermann reactions were obtained, largely in cases of acute respiratory tract infection, in which the Hinton test was consistently negative, and only 7 false positive Hinton tests were obtained.

6 The result of the Hinton test was an accurate indication of the significance of the doubtful Wassermann reaction.

7 The Hinton test was very much more consistent in its reading in the individual case than was the Wassermann

8 In this series, the error in the Wassermann results was 23 per cent, as compared to 13 per cent in the Hinton test

#### CONCLUSIONS

1 In this series of 2120 consecutive cases, the Hinton Glycerol Cholesterol Precipitation test has agreed with the clinical evidence in more cases than the Wassermann reaction.

2 An adequate serologic examination for syphilis should include the Hinton test

#### REFERENCES

- 1 Hinton, William A A Standardized Method of Performing the Wassermann Reaction  
Am J Syph 4 No 4 155 160 October, 1920
- 2 Hinton William A A Glycerol Cholesterol Precipitation Reaction in Syphilis Boston  
M & S J 196 No 24 993 996 June 16 1927

### A METHOD FOR DETERMINING THE LENGTH OF THE FEMORAL NERVE, AND THE LENGTH OF THE REFLEX ARC INVOLVED IN THE KNEE JERK IN THE DOG\*

BY E M MACEWEN M D AND W W TUTTLE PH D IOWA CITY, IOWA

THE structure of the arc involved in the 'knee jerk' reflex has been and still is the subject of many conflicting statements. The arguments, pro and con, have for the most part revolved around the conduction time involved in this reflex. Data for measuring this are lacking since no exact method was available for determining the length of the femoral nerve. In an attempt to obtain exact data on this, the anterior femoral nerves were measured in nineteen cadavers, from the superficial origin of the motor rootlets to the motor point in the rectus femoris muscle. During this work it was found that the length of this nerve corresponded very closely to the distance from the anterosuperior spine of the ilium to the upper border of the patella. The motor point was also found to lie at a mid point between these two landmarks. Tuttle and MacEwen (1928)

The results obtained from the use of these data on the human, were so satisfactory that it was deemed worth while to apply them to some laboratory animal. The dog was selected partly because of its almost universal use, but mainly because the great range in size of this animal afforded ample test for the data.

#### METHODS

A preliminary experiment was performed on three dogs. The anterior femoral nerve on each side was carefully worked out. Starting where it emerged from the psoas major muscle the nerve was traced back to its intervertebral

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foramina The branch to the rectus femoris muscle was isolated and traced to its termination A laminectomy was done and the roots traced back to their superficial origin It was found that by carefully cutting away pedicles, the roots could be brought into almost a straight line with the main trunk of the nerve The branch to the rectus femoris muscle leaves the main trunk shortly after it emerges from the psoas major muscle This is about a centimeter below the level of the inguinal ligament The branch then turns laterally at almost a right angle and is accompanied by the vascular supply to the upper part of the muscle In its course it passes beneath the sartorius to the septum between this muscle and the rectus femoris In this septum it breaks up into three divisions superior, middle, and inferior Each division appears to pass to corresponding one-third of the rectus femoris muscle They enter the deep surface of the muscle and immediately break up into a number of smaller branches In all cases the middle division entered the muscle belly of the rectus femoris at a point which varied from a distance half way between the anterosuperior spine of the ilium and the superior margin of the patella to three and one-half cm proximal to this point As compared with the human, the motor point in the dog cannot be so accurately found without resorting to stimulation Because the termination of this middle branch seemed to correspond so closely to the motor point found for the human, it was called the motor point for the dog's rectus femoris muscle

Under ether anesthesia, the rectus femoris and sartorius muscles were retracted in two dogs Each division of the femoral nerve to the rectus femoris was isolated and stimulated directly When the electrodes were placed on the middle division, a maximal contraction of the entire muscle was obtained The same stimulus applied to the upper and lower divisions resulted in little or no contraction When the electrode was applied to the outer surface of the muscle, over the points of distribution of each division, similar results were obtained This substantiated our idea that the entrance of the middle division represented the true motor point

Five dogs were placed under ether anesthesia, the hair shaved off, the electrode applied to the skin over the rectus femoris muscle, successively from origin to tendon of insertion In all of these, when the electrode was applied over a point on the skin about a centimeter above the mid-distance between the anterior limit of the crest of the ilium and the upper border of the patella, a powerful contraction of the entire muscle was observed

In view of the above facts we felt justified in considering the termination of the middle branch as the motor point In addition it corresponds in relative position to that observed in the human

In ten dogs the distance from the anterosuperior spine on the crest of the ilium to the upper border of the patella was measured with the leg in extension The distance to the motor point as above established was also recorded A complete exposure of the anterior femoral nerve was made on both sides In order to get the middle branch to the rectus femoris muscle in a straight line with the main trunk and exposed roots, the sartorius and rectus femoris muscles were reflected down from their origins We were thus able to get the entire nerve,

from the origin of the motor rootlets of the fourth lumbar, to the motor point in the rectus femoris muscle in a straight line

Table I shows the measurements obtained and a comparison between the length of the femoral nerve and the distance between the anterior limit of the crest of the ilium and the upper border of the patella

## SUMMARY

The dogs used were all adults. In size they varied from large collies to small fox terriers. Inasmuch as the data obtained were so uniform it was deemed unnecessary to extend the series.

TABLE I

A COMPARISON OF THE LENGTH OF THE FEMORAL NERVE WITH THE DISTANCE FROM THE ANTEROSUPERIOR SPINE OF THE ILIUM TO THE SUPERIOR MARGIN OF THE PATELLA

DOG NO	DISTANCE FROM SUPERFICIAL ORIGIN OF MOTOR ROOTS FROM CORD TO MOTOR POINT		DISTANCE FROM THE ANTEROSUPERIOR SPINE OF THE ILIUM TO THE SUPERIOR MARGIN OF THE PATELLA		DISTANCE FROM THE ANTEROSUPERIOR SPINE TO THE MOTOR POINT		DIFFERENCE BETWEEN ACTUAL NERVE LENGTH AND SURFACE MEASUREMENTS	
	RIGHT LEG	LEFT LEG	RIGHT LEG	LEFT LEG	RIGHT LEG	LEFT LEG	RIGHT LEG	LEFT LEG
	CM	CM	CM	CM	CM	CM	CM	CM
1	20	20	20	20	6.5	6.5	0	0
2	22	22	21	21	7	7	1	1
3	18	18	18	18	9	9	0	0
4	24	24	24	24	10	10	0	0
5	21	21	21	21	9	9	0	0
6	13.5	13.5	13.5	13.5	8	8	0	0
7	15	15	15	15	7	7	0	0
8	21	21	21	21	9	9	0	0
9	22.5	22.5	23	23	9	9	0.5	0.5
10	17.5	17.5	18	18	8	8	0.5	0.5
11	16	16	16	16	7	7	0	0
12	18.5	18.5	18.5	18.5	7.5	7.5	0	0

In twelve dogs, the data are comparable to those found for the intact human and warrant similar conclusions as follows:

1 The length of the femoral nerve from its superficial origin in the cord to its end plate in the rectus femoris muscle is equal to the distance from the anterosuperior spine of the ilium to the superior border of the patella.

2 The motor point of the rectus femoris muscle in the dog is not constant as in the case of the human. In most cases it is proximal to the middle of the muscle and can be located accurately only by the method of stimulation.

3 The length of the reflex arc involved in the knee jerk of the dog is determined by doubling the distance from the anterosuperior spine of the ilium to the superior margin of the patella and adding to this the distance from the point of stimulation to the motor point of the femoral nerve in the rectus femoris muscle.

## REFERENCE

Tuttle W W and MacEwen E M. A Method of Determining the Length of the Femoral Nerve and For Locating the Motor Point of the Rectus Femoris Muscle in the Intact Human Body, *Anat Rec* 37 No 3 317-319 1928.

# THE USE OF BUFFER CITRATE SOLUTION AS A DILUENT AND PRESERVATIVE FOR RED BLOOD CELLS\*

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AFTER observation of the effect of a buffered citrate solution, as used by AD<sup>1</sup> Benjamin Jablons<sup>1</sup> upon the red blood corpuscles after intravenous injection, it occurred to me to study the properties of the same solution as a laboratory agent for dilution and preservation of red blood cells. The following is a brief resumé of the studies and observations along this line.

It was observed that whole blood taken from the vein and mixed with this solution in varying proportions from that of equal parts to a concentration of 1 to 200 failed to show any appreciable effect on either the red or white cell components of the blood. After eight weeks exposure at room temperature in this solution, there was no evidence of any disintegration of the red cells or any appreciable change in the white cells. The fact that no chemical change takes place in the protein molecule of either the red or white blood cells, is proved by the following observation.

The addition of a 1 per cent solution of phenol red to the solution containing the blood, even after eight weeks exposure and contact at room temperature, showed no change in the  $P_H$  or hydrogen-ion concentration of the solution containing the blood. It was also found that blood cell counts did not vary after a lapse of eight weeks, excepting for the permissible error due to the personal equation. The composition of the solution is as follows:

Sodium Citrate .....	20 grams
Dibasic Potassium Phosphate .....	2 grams
Sodium Chloride .....	3 grams
Doubly distilled water .....	1000 grams

The  $P_H$  should be approximately 7.7. It has also been found that where it is desirable to make counts of the white blood cells, that the addition of methylene blue is somewhat of value. This dye when exposed to room temperature for eight weeks, however, fades somewhat although the blood cells themselves retain their color. Examination of the blood after eight weeks contact with this solution at room temperature, shows the red cells to be unaffected and the white cells completely impregnated with the dye.

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<sup>1</sup>Jablons, Benjamin. Thrombo Angitis Obliterans. Inter Clinic 111, 193, Sept. 1925.

## THE PHENOMENON OF ALCOHOLIC ANTIGEN PRECIPITATION IN LUETIC SERUMS\*

BY B S LEVINE, PH D, WASHINGTON, D C

BORDET and Gengou<sup>1</sup> published a paper in 1901, the purpose of which was to prove the functional unity of complement Wassermann, Neisser and Bruck aptly utilized the principle of the unity of complement in the diagnosis of syphilis in inoculated monkeys Later, in association with Schuecht,<sup>2</sup> the same authors extended their new method for the diagnosis of syphilis to human beings Following the discovery of these authors, Michaelis<sup>3</sup> described a precipitation reaction for the diagnosis of syphilis in which he used serum, a watery extract of syphilitic liver, and normal saline He considered the reaction specific, believing, as did all serologists of his time, that the syphilitic liver extract contained the specific antigen

Numerous modifications and new precipitation procedures for the diagnosis of syphilis have since been proposed Not until recently, however, have any of them proved of sufficient value to be generally adopted Kahn<sup>4</sup> had demonstrated that by paying heed to certain fundamental conditions, the precipitation of alcoholic antigen with a syphilitic serum could be made to serve the same purpose as does the Wassermann reaction

The value of the laboratory procedure for the diagnosis of syphilis, whether by the complement-fixation or by alcoholic antigen precipitation, has been established empirically The tests, therefore, must still be looked upon as mere adjuncts to the clinical diagnosis of any case under consideration Nevertheless, sound theoretical reasoning must underlie such laboratory procedures, if the clinician is to depend upon them at all The gross or basic principles of the Wassermann reaction are at present understood Antibody, by attaching itself to the alcoholic antigen, sensitizes it, enabling complement to come into play and to complete the formation of the antigen antibody complement complex The phenomenon of antigen precipitation, however, has remained unexplained It is true, as mentioned above, that Michaelis, as far back as 1907, expressed his belief in the specificity of the reaction, and that Meenecke<sup>5</sup> tried to show that in his third modification of antigen precipitation he was dealing with a phenomenon similar to complement fixation Yet, neither offered definite experimental proof in support of their beliefs

Jacob (Else)<sup>7</sup> added complement and the hemolytic system to the completed Sachs Georgi test, and has concluded from the results obtained that the Wassermann and the Sachs Georgi reactions are essentially the same

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However, the author failed to account for the disagreements in the results obtained by such a procedure of experimentation

Kahn<sup>8</sup> had studied the identity of his antigenic precipitate and of complement fixing substances by a similar procedure. Such a procedure for the study of the two reactions is considered by us inappropriate, and the results inconclusive. There is, in the first place, a certain percentage of disagreement cases which call for an explanation. In addition, the experiments show merely that the two reactions are coincidental in a large percentage of cases, but fail to show analytically that the factors underlying the two reactions are the same.

Jacobstahl<sup>9</sup> showed that the precipitate produced by the precipitation reaction fixes complement, and that the supernatant fluid, if free from sediment, does not fix complement. From this he inferred the identity of luetic precipitins with complement fixing substances.

Keining,<sup>10</sup> on the other hand, having made a comparative study of the results obtained by the Sachs-Georgi precipitation and by the complement-fixation procedures, found that frequently a positive Sachs-Georgi yields a negative superimposed Wassermann, whereas, on the other hand, a negative Sachs-Georgi may result in a positive Wassermann. He, therefore, came to the conclusion that the antigen precipitation and complement-fixation reactions depend upon different serum constituents.

On the basis of discrepancies in the results obtained by the Kahn precipitation and by the complement fixation procedures, Kilduffe<sup>11</sup> also suggests a difference either in the mechanism or in the substances involved in the two diagnostic procedures.

Not until the true nature of the antigen precipitation by luetic serum is well established, will it be possible to intelligently discuss the results of the precipitation and of the complement fixation on a comparative basis. With a view to obtaining first hand information on the subject under consideration, I have made several series of elaborate experiments described herein.

A number of serums have been tested by the following three methods: (1) U. S. Veterans' Bureau<sup>12</sup>, (2) Kolmer<sup>13</sup>, (3) Kahn<sup>14</sup>. The racks containing the Kahn tests of the serums which proved positive by the three tests mentioned were properly marked and placed in the ice box overnight. They were subjected to prolonged centrifugation in order to throw down the precipitates. The supernatant diluted serums were poured off into another set of tubes marked to correspond with the original. The two sets of the tubes were placed into separate racks marked "P," for precipitate, and "S" for supernatant fluid. To each tube of the "P" set, one mil of 0.85 per cent saline was added, and the precipitates resuspended by agitation. The contents of the tubes of both sets were tested by the complement-fixation procedure. It was found that the complement remained free in the supernatant fluids, but was completely fixed by the precipitates. This was in agreement with the results of Jacobstahl.

From a consideration of the known sero-immunologic principles, complement fixation and resulting inhibition of hemolysis occur only in the union

of antigen with antibody. And since only one kind of antibody is capable of uniting with the same antigen, the experiment affords reason to suspect that the precipitation of the antigen was caused by the antibody present in the serologically positive serums.

In the above experiments some of the supernatant fluids containing the serum remained in association with the sedimented precipitates. To eliminate the influence which might have been exerted by the traces of serum upon the fixation of the complement a set of positive serums had been treated as described above. The sedimented antigenic precipitates, then, had been washed with saline and centrifuged. This step had to be carried out with extreme caution. Washing the sediment with saline may cause it to become resuspended in the wash saline in an inseparable condition. The tubes containing the sediment, the wash solution, and the original supernatant fluid were tested by the complement fixation procedure. Complete fixation of the complement was manifest in the tubes containing the washed precipitate and complete hemolysis in the tubes containing the original supernatant fluid and wash saline. The fixation of complement in the tubes containing the sediment in the first experiment, therefore, was not due to the presence in those tubes of traces of the positive serum. It was caused by the absorption of the complement by the sedimented antigen antibody complex.

The results of the above experiment demonstrate that alcoholic antigen is precipitated by so called positive serums as a consequence of the formation of an antigen antibody complex which is capable of absorbing complement. Since, as stated above, the antigen used in the complement fixation tests was prepared practically the same way as the antigen used in connection with the precipitation tests it follows that the antibody involved in the antigen precipitation reaction is identical with the antibody concerned in the Wassermann reaction. It appears valid, therefore, to conclude that antigen precipitation demonstrates the same immunologic state of the serum tested as does complement fixation.

Similar results by the use of a similar procedure were obtained by Stuhmer and Merzweiler<sup>1</sup> in connection with a study made by the authors on the Sachs Georgi and the Wassermann reactions. They concluded, as a result of their experiments, that contrary to the opinion of Kenning,<sup>10</sup> "dislipidization" is not the factor which inactivates the "reagin" but that the reagin is removed from the serum extract mixture by the antigenic precipitate.

In a critical discussion on the validity of testing the antigenic precipitates by procedures similar to those used by us and other investigators and of the conclusions drawn from the results obtained Kafka<sup>18</sup> concludes that such experiments do not demonstrate a fixation of complement in the same sense as it is understood to occur in the Wassermann reaction, but that a purely physical destruction of the complement, incident to its adsorption by the suspended precipitate, takes place. Rothman<sup>17</sup> had used barium sulphate and copper hydroxide in saline solution, and by appropriate precipitation and dilution suspended them so that agglutinoscopically they resembled a straight Sachs Georgi reaction. He found that such precipitates fixed complement,



while the filtrates did not fix complement. He concluded, therefore, that the hemolytic inhibition caused by the precipitated serum-extract mixtures indicated no specific complement fixation. Like Kafka, he considered that such inhibition was due to the physical destruction of the complement caused by its adsorption by the macroscopic precipitate.

The error of Rothman's conclusion evidently arises from the fact that he deals in his experiments with strictly nonspecific precipitates. However, to prove his and Kafka's errors, experimentation was required. In connection with the study of the principles controlling alcoholic antigen precipitation, I have found the following. When cholesterinized alcoholic antigen is precipitated with an equal volume of 0.85 per cent salt solution, placed in proper quantities into serologic tubes, followed by 0.15 c.c. of serum, shaken for three to five minutes, as prescribed by Kahn, and then 1.0 c.c. of distilled water instead of saline is again added, the tubes shaken, and allowed to rest overnight, the alcoholic cholesterinized antigen is precipitated from the negative as well as from the positive serums. By centrifugation the removal of the supernatant fluids is accomplished with ease and completeness.

Having established the constancy of the phenomenon just described, the following sets of experiments were carried out. Negative and positive serums were subjected to reaction with antigen as per the Kahn procedure. The antigen was, then, precipitated out with distilled water as described above. The tubes were allowed to stand overnight in the ice box. The following day, they were thoroughly centrifuged, and the supernatant fluid removed from the sediments. To the tubes containing the supernatant fluids, 8.5 per cent saline was added to the extent of 0.1 c.c. per 1.0 c.c. of distilled water, to restore the original tonicity. The tubes containing the precipitates and the supernatant fluids, then, had been tested for complement fixation. No complement was absorbed by any of the supernatant fluids nor by the precipitates obtained from the negative serums.

In another set, a number of negative serums were treated with cholesterinized alcoholic antigen exactly as described above. After having separated the precipitates from the supernatant fluids, one-half of the precipitates were resuspended in 0.85 per cent saline, while to the precipitates of the other half appropriate quantities of a number of positive serums were added. The tubes were well shaken for three to five minutes, and the cholesterinized antigen reprecipitated from the positive serums with distilled water. The sediments thus obtained were again separated from the supernatant fluids. After proper adjustment for tonicity, all the supernatant fluids and the precipitates had been tested for complement fixability. The complement remained free in all the tubes containing the supernatant fluids as well as in the tubes containing the precipitates obtained from the negative serums which had not been subjected to any further treatment. The complement was completely fixed by the precipitates originally obtained from the negative serums and later treated with positive serums.

The results of these sets of experiments show conclusively that the mere physical condition of the antigenic precipitate does not sensitize it to the

absorption of complement Only in the union with syphilitic antihody does the precipitated antigen fix complement

To further substantiate this conclusion, the following experiment was carried out A 0.5 per cent alcoholic solution of pure cholesterol was prepared Two ml of this solution were mixed with an equal volume of 0.85 per cent saline A flocculent precipitate resulted Five hundredths cc of the cholesterol saline mixture was placed into each of a series of serologic tubes Fifteen hundredths cc of each of a number of positive and of negative serums, then, had been placed into the tubes, and the racks well shaken for three to five minutes One ml of distilled water, then, was added to each tube, the racks again shaken and left stand in the ice box overnight The following day the tubes were centrifuged, the supernatant fluids removed from the precipitates, and after proper adjustment for tonicity, the supernatant fluids and the precipitates had been tested for complement fixation

The complement remained free in the tubes containing the supernatant fluids from the negative serums and in the tubes containing the precipitates from both, the negative as well as the positive serums The complement was completely fixed in the tubes containing the supernatant fluids obtained from the positive serums The above experiments vividly show that nonspecific flocculent colloidal precipitates obtained from either positive or negative serums do not fix complement, because they do not remove with them the luetic antihody from the positive serums It is, therefore, valid to conclude that the precipitates yielded by the Kahn positive serums, possessing complement fixing properties, represent the antigen antihody complex Furthermore, since the removal of the precipitates renders the supernatant fluids Wassermann negative it is likewise valid to infer that the same antibody that is responsible for the fixation of the complement in the Wassermann reaction, is responsible for the precipitation of antigen by the Kahn and other precipitation procedures

#### CONCLUSIONS

- 1 The phenomenon of antigen precipitation in the Kahn and in similar reactions is a manifestation of the formation of an antigen antibody complex
- 2 The same antihody that is concerned in the complement fixation test is responsible for the phenomenon of antigen precipitation in luetic serums
- 3 Antigen precipitation in syphilis, therefore, is a direct manifestation of the first step of the Wassermann reaction, namely of the union of alcoholic antigen with the luetic antibody
- 4 The difference in the results obtained by the various complement fixation and antigen precipitation procedures, therefore, is not due to any difference in the principles upon which the reactions depend or of the primary substances involved It is the difference in the concentration of the complex reagents used, a factor which profoundly affects the balance of the colloidal system under study, that causes the discrepancies

This phase of luetic serology will form the subject matter of a following paper

## REFERENCES

- 1 Bordet, J, and Gengou Sur l' existence des substances sensibilisatrice dans la plupart, des serums antimicrobiens, Ann de l'Inst Pasteur 15 289, 1901
- 2 Wassermann, A, Neisser, and Bruck Eine serodiagnostische Reaktion bei Syphilis, Deutsche med Wehnschr 32 745, 1906
- 3 Wassermann, A, Neisser, Bruck, and Schuchet Nachweis spezifischluetischen Substanzen durch Komplementverankerung, Ztschr f Hyg u Infektionskr 30 1769, 1906
- 4 Michaels, L Precipitinreaktion bei Syphilis, Beil klin Wehnschr 44 1477, 1907
- 5 Kahn, R L Serum Diagnosis of Syphilis by Precipitation, Williams and Wilkins Co, Baltimore, 1925
- 6 Memmeke, E Ueber die dritte Modifikation Munchen med Wehnschr 56 932, 1919
- 7 Jacob, Elsc Zur Frage der praktischen Brauchbarkeit der Ausflockungsreaktion nach Sachs Georgi, Dermat Ztschr 31 part 6, 1920
- 8 Kahn, R L et al Identity of Precipitin and Complement Fixing Substances in Syphilitic Sera, Proc Soc Exper Biol & Med 24 775, 1927
- 9 Jacobstahl, E Die Wa R eine Prezipitationsreaktion, Arztl Verein Hamburg, Nov 16, 1909
- 10 Keming, E Ueber die kombinierte SG Reaktion, Deutsche med Wehnschr 47 157, 1921
- 11 Kilduffe, R A Ref U S Veterans' Bureau Clin Bul 18, 1927
- 12 Clin Bul No 10, U S Vet Bureau, 1926
- 13 Kolmer, J, and Boerner Laboratory Diagnostic Methods, D Appleton and Co, New York, 1926
- 14 Kahn, R L Loc cit
- 15 Stuhmer, A, and Merzweiler Ueber eine kombinierte Sachs Georgi Wassermannsche Reaktion Deutsche med Wehnschr, p 559, May 19, 1921
- 16 Kafka Sitzungsber, Hamburg Aerztl Verein, 1921, Ref Munchen med Wehnschr p 258, March 3, 1921
- 17 Rothman, Stephan Zur Kombination der Sachs Georgi und Wassermannsche Reaktion, Deutsche med Wehnschr, p 953, Aug 15, 1921

## AN ALKALI RESISTANT STOPCOCK\*

BY F C KOCH, PH D, CHICAGO

STOPCOCKS made of black, hard rubber have been used in glass burettes containing 10 per cent and 40 per cent sodium hydroxide respectively and have been found satisfactory in every respect. There never has been any indication of the stopcocks sticking or any suggestion of developing leaks. Such stopcocks are relatively easily prepared on the usual lathe equipment found in laboratory shops.

\*From the Department of Physiological Chemistry, The University of Chicago  
Received for publication July 19 1928

## A NEW SET OF POTASSIUM DICHROMATE STANDARDS FOR DETERMINATION OF THE ICTERUS INDEX\*

By CHARLES C. FARHAUGH AND GRACE MEDES MINNEAPOLIS MINN.

THERE is a growing conviction among those concerned with the estimation of quantitative variations in serum bilirubin, that the determination of the icterus index offers a more satisfactory method than the more laborious and less accurate quantitative modification of the van den Bergh<sup>1</sup>

The determination of the icterus index is originally worked out by Meulengracht, was accomplished by dilution of the plasma until it matched a given standard consisting of a 1 to 10 000 aqueous solution of potassium bichromate

A number of modifications of this method have been made by other workers. The most widely used modification seems to be that in which the color comparison is made in an ordinary colorimeter. The same standard (1 10,000 solution of potassium bichromate) is used. The serum is diluted with physiologic salt solution until it nearly matches the standard, the colorimetric reading is made, and the factor  $\frac{\text{standard reading}}{\text{unknown reading}}$  is multiplied by the dilution. This method can be employed only in laboratories where colorimeters are available, and it has the disadvantage that at least 5 cc. of serum are needed to make a reading.

A more satisfactory method is that proposed by Murphy<sup>2</sup> in which a series of test tubes are used filled with bichromate solutions, the concentrations of which are as follows: 1, 2, 5, 10, 15, 20, 25, 50, 75 and 100 parts of potassium bichromate in 10,000 parts of water corresponding to indices of 1, 2, 5, — — — 100. A quantity of serum is placed in a similar glass tube and by comparison its index value is determined without dilution.

There are two weaknesses in this method. The first is that it attempts to cover the whole range of index values in a single series and leaves too large gaps between the tubes. For instance it is generally agreed that the serum of normal persons gives an index value of 4 to 6 and that when skin icterus becomes just visible, the reading is 15. The range between these two values 5 to 15, has been designated as that of "latent jaundice." Obviously it is in this range that icterus index readings are most valuable. The series of standards as arranged by Murphy gives only a single value within this range, and is therefore not adapted for quantitative estimations, the first standard above the normal being 100 per cent greater than normal.

The second weakness of the Murphy method lies in the fact that in the standards of highest concentration the yellow color becomes so intense that variations are difficult to observe and the more nearly the color approaches saturation, the greater the error. In a series of investigations carried out in

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If desired, an attachment may be added for refilling the apparatus without dismantling it. A mercury leveling bulb is connected with a Y-tube, one arm of which passes through the rubber stopper to the bottom of B. By lowering the leveling bulb the mercury is siphoned from B and at the same time A is refilled with the injection fluid by drawing it in through Z. The second arm of the Y-tube from the mercury leveling bulb may be fused to the burette just above the stopcock and used for refilling the burette with mercury. During the injection both arms of the Y-tube are of course closed. With such an

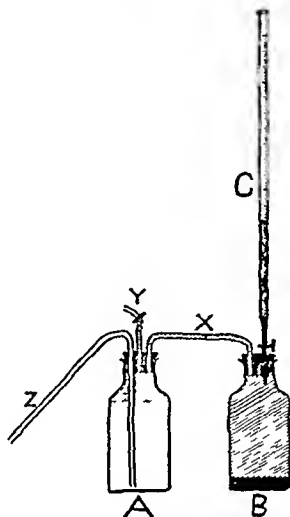


FIG 1

attachment the system resembles the Trendelenburg apparatus for recording blood pressure.

The use of bottle B and of paraffin oil is merely for the purpose of preventing the injection fluid from coming in contact with mercury. If such is considered unimportant the mercury may be allowed to drop directly into A with tubing Z passing only to the lower surface of the stopper.

The advantages of the system are its simplicity, the slow constant rate of injection possible, and the ease with which the rate may be varied.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE M.D., ABSTRACT EDITOR

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# RAPID AND ACCURATE BLOOD CHEMISTRY DETERMINATIONS\*

BY W G GAMBLE, JR, M D, CHARLESTON, S C

BLOOD chemistry determinations are now part of the routine laboratory procedures. This has increased the work of the laboratory personnel without materially increasing its numerical strength. Hospitals and physicians request emergency blood chemistry determinations, such as, blood sugars, and

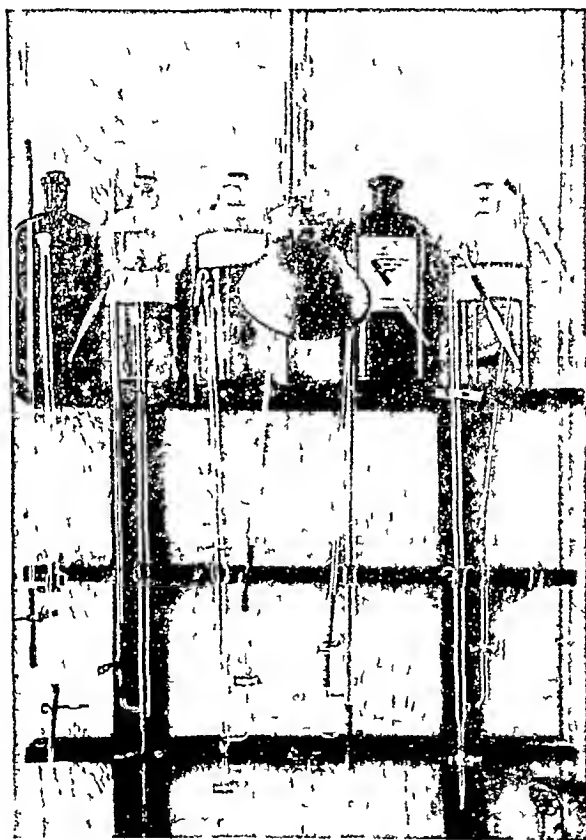


Fig 1

they desire reports in the shortest possible time. These determinations are at best time consuming, and especially is this so when a number of specimens are submitted.

Fig 1 represents a device which may not be new, but as far as I know it has not been pictured. It is especially adaptable to the Folin-Wu methods and decreases the time element on single, as well as on numerous specimens.

\*Received for publication July 24 1928

# A NOTE ON THE INTRAPERITONEAL METHOD OF INDUCING ANESTHESIA WITH BARBITAL NA IN THE DOG\*

By C A JOHNSON,† CHICAGO, ILL

IN 1922 Parsons and Tatum<sup>1</sup> described the oral use of barbitol as an anesthetic agent for dogs. Since that time Percy and Weaver indicated and discussed the various methods of inducing anesthesia with this drug and some of its derivatives.

For the past two years we have used barbitol Na as an anesthetic in acute dog experiments and have found it very satisfactory when given by the intraperitoneal route in similar doses suggested by Percy and Weaver (250 mg per K). In most cases anesthesia occurred in from fifteen to thirty minutes until suddenly we had a great number of failures, i.e., with the same doses per K body weight, anesthesia would not occur for several hours if at all.

At first we attributed these failures to the drug being of poor quality and thus led us to obtain another preparation from a different drug house. Using this, we still continued to have poor results. It then occurred to us that the site of injection had been changed. In the successful cases the upper abdominal quadrants had been used for injection while in the unsuccessful ones we had used the lower quadrants. Further observations as indicated in Table I which contains the more or less quantitative data in support of the contention.

TABLE I

WT OF ANIMAL	AMOUNT OF DRUG PER F	SITE OF INJECTION	ANESTHESIA IN
15 K	250 mg	Just below the Xiphoid	26 minutes
11 K	250 mg	Just below the Xiphoid	24 minutes
15 K	250 mg	Just below the Xiphoid	16 minutes
15 K	250 mg	Right lower quadrant	2 Hours and 16 min
11 K	250 mg	Left lower quadrant	1 Hour and 42 min

It has been stated and observed clinically that absorption from the upper abdominal cavity is more rapid. We can interpret these data on the basis of a more rapid absorption by the visceral and parietal peritoneum of the upper abdominal cavity.

We have written this note, hoping that the suggestion for the administration given above will save time and prevent disappointment of those investigators who wish to use the intraperitoneal method of inducing a satisfactory anesthesia with barbitol Na.

## REFERENCES

- 1 Tatum and Parsons J LAB & CLIN MED 8 64 1922 23
- 2 Percy and Weaver J LAB & CLIN MED 12 1071 No 2 1927

From the Physiological Laboratories of the University of Chicago

Received for publication August 4 1928

†Aided by a grant from the Billings Medical Club Chicago Ill



## RAPID AND ACCURATE BLOOD CHEMISTRY DETERMINATIONS\*

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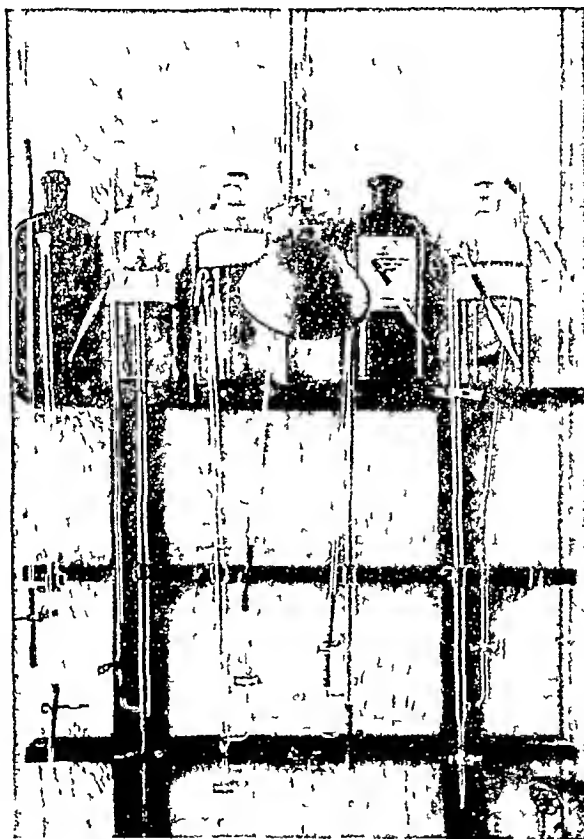


Fig 1

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Each apparatus consists of a two gallon jug connected by a siphon glass tubing and suitable pinchcocks, to an automatic refillable burette of 50 cc capacity graduated accurately to 0.1 cc. All air vents are protected by trap tubes containing soda lime. The other details are self evident. For blood sugar determinations a battery of five is used. First, Container of distilled water. Second, 10 per cent sodium tungstate. Third,  $2/3$  N sulphuric acid. Fourth, Folin copper tartrate solution. Fifth Folin molybdate acid solution. Others for Nessler's solution etc., may be added so this method may be used in other chemical determinations, for in our hands, it has proved readily adaptable.

Slow pipetting is avoided and rapid precipitation accomplished. We find it possible to complete blood sugar determinations in twenty five minutes.

This method has been checked repeatedly and the results are practically the same.

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## A SIMPLE METHOD FOR SLOW INTRAVENOUS INJECTIONS\*

By RALPH G. SMITH, M.D. † AND WALTER E. GOWER, M.A., CHICAGO, ILL.

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**I**N LABORATORY work it is sometimes desired to make continuous intravenous injections at a very slow rate. For this purpose the apparatus described below has been adopted with satisfactory results. By using the passage of mercury through a stopcock as the controlling factor it is possible to attain a much slower rate than by the direct injection of an aqueous solution from a burette. By the method to be described the injection may be slowed to a rate of 1 cc. in twenty minutes.

Fig. 1 shows the essential features of the apparatus. It consists of a burette with a stopcock and two similar bottles, A and B, of such a size that one will contain the volume of the fluid to be injected. They are assembled with bored rubber stoppers and tubing as represented in the diagram. In preparation for use A is filled with the solution to be injected. B with paraffin oil, and the burette with mercury. The mercury is allowed to drop into B thus displacing the oil through X into A. Air bubbles are driven from B in front of the oil and are forced from the system through Y which remains open while Z is closed. On closing Y and opening Z, which is connected with a hypodermic needle, the injection fluid is forced from the latter at the desired rate which is controlled by the burette stopcock. The volume of fluid injected is determined by the fall of mercury in the burette. As the injection proceeds the rate will be somewhat slowed by such a fall, due to a decrease in pressure. This, of course, may be rectified by readjusting the stopcock or by refilling the burette at frequent intervals. However if it is essential that the rate remains constant throughout, mercury may be allowed to drop from a second burette into the first burette at such a rate that the level in the latter remains practically constant. The volume of fluid injected is then determined by reading both burettes.

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From the Laboratory of Pharmacology, The University of Chicago, Chicago, Illinois.  
Received for publication September 9, 1918.  
†Fellow in Medicine of the National Research Council.

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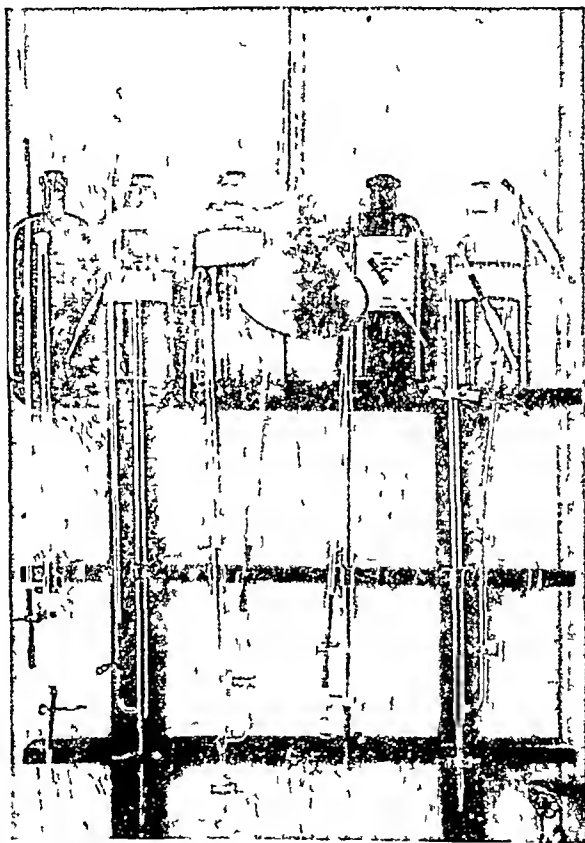


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The successful results with a medium composed of seven parts of normal salt solution and one part of inactivated human blood serum demonstrate that none of the

chemicals in either Locke's or Ringer's solution, with the exception of sodium chloride, were essential in the media used in the survey and that the continued cultivation of *Entameba histolytica* over an indefinite period of time is possible in this simple medium, as shown previously by Craig

The formula for the medium follows

The Locke solution in this medium has the following formula

Sodium chloride	9 00 gm
Calcium chloride	0 24 gm
Potassium chloride	0 42 gm
Sodium bicarbonate	0 20 gm
Dextrose	2 5 gm
Distilled water	1000 cc

The method of preparing this medium follows

The Locke solution is filtered and autoclaved at 15 pounds pressure for fifteen minutes and then allowed to cool. Four whole eggs are emulsified in 50 cc of the solution. The mixture is tubed and coagulated in slants, and to each slant is added sufficient inactivated horse serum diluted with 8 parts of the Locke solution to cover the whole or part of the slant. The tubes are then incubated at 37° C for several days to test for sterility. If sterile, they are kept in the incubator at 37° C until used.

#### LOCKE SERUM MEDIUM

The Locke solution used in this medium is the same as that used in the preparation of Locke Egg Serum medium, with the exception that 1 gram of dextrose is used instead of 25 gm. The solution is filtered and autoclaved as described above. There is then added one part of human blood serum inactivated by heating in a water bath at 50° C for one half hour, to each 7 parts of the Locke solution. After adding the blood serum, the mixture is thoroughly shaken and filtered through a Mandler or Berkefeld filter. It is sometimes necessary to filter the mixture through two or more candles in order to obtain a perfectly clear filtrate. After filtration, the medium is tubed, placing from 5 to 10 cc in each tube, and incubated at 37° C for several days to determine sterility. If found sterile, the tubes are kept in an incubator at 37° C until used. The reaction of the medium does not need adjustment.

#### TYPHOID FEVER. Otani Reaction. Study of Typhoid Fever, Hilario, S, and Ira, F. Rev. Filmed by farm 16 244, November, 1925

Otani's reaction is based upon phagocytosis and characterized by intensification of opsonic capacity under the stimulus of bacterial antigens.

According to Otani the normal phagocytic index is not over 10 per cent. He finds, in typhoid fever, tuberculosis, and dysentery, an increased index strictly specific for these organisms but not for related organisms.

He claims that it has a great advantage over Widal's reaction because it becomes manifest much sooner than the latter and is more sensitive and yet there are no false reactions. In typhoid fever Otani's reaction appears on the second or third day of the disease, that is, within the first week, being in this respect as rapid and efficient as hemo culture. The technique is simple and consists in placing 4 or 5 drops of blood in a test tube which contains a drop of sodium citrate solution and shaking the tube until it is well mixed. A small quantity of the liquid is taken with a fine pipette and an equal amount of a fine emulsion of the bacillus of the disease to be studied added to it. These two solutions are mixed in a capillary tube by the ordinary method and the tip of the tube closed, after which it is put in an incubator at 37° or in a water bath at 38°. After ten minutes it is removed and the tip of the capillary tube broken off and a drop of its contents placed on a slide, making a smear. When the smear is dry it is stained with Wright's, Otani's or Giemsa's stain. The leucocytes take the basic color and the microorganisms the violet color. It is examined with the immersion lens, the leucocytes which contain bacteria being put down in one column and the leucocytes free of bacteria in another, at least 100 leucocytes being counted, the exactness of the results

being proportional to the number of leucocytes counted. Any average larger than ten is considered positive and any less than or equal to ten negative. The blood should be fresh as the leucocytes lose vitality if they are kept for some hours, even if it is kept at body temperature. The emulsion should be fine and made of a culture not more than twelve to twenty four hours old. The saline solution must be isotonic. The sodium citrate solution is a 5 per cent solution in physiologic salt solution. All the apparatus used should be free of fat and alkali, as these substances slow phagocytosis.

**BILE ACID IN BLOOD** A New Colorimetric Method for the Quantitative Determination of Bile Acid Salts in the Blood Sallard P. *Biochem Ztschr* 173 440 June 25, 1926

#### REAGENTS

- |   |                                                 |         |
|---|-------------------------------------------------|---------|
| 1 | 1 per cent aqueous solution ferric chloride     | 1000 cc |
|   | Conc HCl (sp grav 1.13)                         | 05 cc   |
|   | This solution is stable if kept in dark bottles |         |
| 2 | Sulphosalicylic acid cp                         | 20 gm   |
|   | Distilled water                                 | 100 cc  |
| 3 | Standard Solution                               |         |

Place 0.44 gm of cp ferric ammonium sulphate in a liter graduated flask, add 950 cc of distilled water 1 cc of concentrated HCl (sp grav 1.13) and make up to 1000 cc.

Filter. Transfer 100 cc to a graduated liter flask, add 0.9 cc of HCl (sp grav 1.13) and make up to 1000 cc. This solution is stable for two to three months.

To 5 cc of this last solution in a test tube add 0.2 cc of the 20 per cent sulphosalicylic acid solution. Allow to stand ten minutes and place in the colorimeter. This amount gives a rose red color equivalent to that given by 0.0625 mg of sodium glycocholate.

#### METHOD

Secure blood by venipuncture and exsulate. Centrifuge and add 5 cc of the plasma drop by drop to 250 cc of absolute alcohol with constant agitation of the flask. Boil while shaking over a water bath for three minutes and filter. Rinse the beaker or flask with 3 portions of hot absolute or methyl alcohol and add the rinsings to the filter.

Distil off the alcohol until the volume is reduced to 10 to 15 cc. Cool and add 300 cc of ether slowly, with constant agitation. Allow to stand until turbidity clears. Decant the alcohol ether and wash the precipitate with 3 or 4 changes of ether. Decant the ether and evaporate to dryness on a water bath.

Dissolve the precipitate in 5 cc of cold distilled water. Centrifuge if necessary to clear, and add 0.3 to 0.5 cc of ferric chloride solution. Place in the incubator until the fluid above the precipitate is clear and transparent.

Centrifuge and wash the precipitate twice with distilled water. After the last centrifuging add 5 cc of water, shake, and pour into a large test tube. Wash the centrifuge tube with 5 cc of alcohol and add to the large tube. Mix and add 5 cc of chloroform. Mix and add 0.2 cc of 20 per cent sulphosalicylic acid. Mix and add 5 cc of water containing 0.5 cc HCl (sp grav 1.13) per 1000 cc. Shake and set aside until the water (colored red) and the chloroform have separated.

Evaporate the chloroform by pouring warm (50°) water over the tube.

Compare the watery red solution with the standard in a colorimeter.

Calculation  $x = \text{unknown}$

$z = \text{standard content of bile salt (0.0625 mg)}$

$H = \text{height of standard (25 mm)}$

$K = \text{unknown solution}$

$$x = \frac{H}{K}$$

Carrying out the multiplication, 15625 must be divided by the number of millimeters read corresponding to the unknown quantity.

With this technique bile acid salts can be demonstrated in small amounts in normal blood.

## REVIEWS

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Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building,  
Richmond, Va

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### *The Young Man and Medicine*<sup>\*</sup>

THE successful business man has recognized for years that the average college student upon graduating knows not what he wishes to do and is equipped for no particular line of life work. University authorities are gradually coming to realize these same facts. One of the most progressive signs of the times in university education is the radical step which has just been taken by the President of the University of Michigan in the formation of a two year academic course at the end of which time the student must have made a decision as to preferential studies.

And now we have orientation courses in which the student learns briefly of the general character of the various professions, and we have series of lectures by outstanding men describing the fields covered in the work in which they find their special interests.

The next logical development would be the appearance of a series of articles or books which will be available for the young man and in which he may read of the work which is done in each profession and be guided in reaching his own decision.

The vocational series edited by Dr E Hershey Sneath of Yale consists of a series of twelve volumes. These cover the fields of law, the ministry, teaching, medicine, journalism, banking, business, mechanical, electrical and civil engineering, farming, and government service.

No two men would write a volume on *The Young Man and Medicine* from exactly the same point of view. Dr Barker as he usually does has made his volume tremendously interesting and thoroughly complete although perhaps a little too technical to inspire the enthusiasm of a young college student.

We should have liked to have seen a little more of the history of medicine and quite a lot more of stimulating biographic sketches of the great men of the profession. These personal differences of opinion as to how such a book should be written, aside however, it is a volume which should be carefully read by every young man who is considering the profession of medicine as a life career.

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### *Percival's Medical Ethics*<sup>†</sup>

THOMAS PERCIVAL lived between 1740 and 1804. We owe to him the foundation of our present system of medical ethics. Professor Leake has reprinted and reviewed critically Percival's principles and compared them with the original oaths of Hippocrates and the principles of medical ethics as they have been propounded by the American Medical Association. It is well that this has been done by a man who is neither in practice nor a physician but who is well acquainted with the customs of the profession. He points out the

<sup>\*</sup>*The Young Man and Medicine*. By Lewellys F Barker MD LL D Professor Emeritus of Medicine Johns Hopkins University. (Vocational Series edited by E Hershey Sneath Ph D LL D Yale University) Cloth Pp 202 The Macmillan Company New York, 1928.

<sup>†</sup>*Percival's Medical Ethics*. Edited by Chauncey D Leake Associate Professor of Pharmacology University of Wisconsin and Lecturer in Pharmacology University of California. Cloth Pp 291 The Williams & Wilkins Company Baltimore Md 1927.

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NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

weak points in our system, probably the greatest criticism of which is that the principles are designed more for the protection of the physician than of the patient

True, there are instances in which the matter of so called ethics is much over done sometimes unfortunately not to the benefit of the patient but after reading Professor Leake's criticisms and rendering the present day principles of medical ethics the reviewer comes to the conclusion that this would be a sad profession indeed and that the patient would on the whole be far worse off did we not possess a concrete system very much in its present form. Possibly we have reached the point where it would be well for the American Medical Association to review its principles altering them where desirable in the interests of the patient but we venture to predict that there would be relatively few changes. The trouble is not with the principles as much as with their practice. The conscientious physician applies the spirit of the principles as he should. The ignorant physician is helped to remain along the straight path by their existence. The unscrupulous physician would not be influenced by even the most perfect system of medical ethics. The trouble is that the purist who has had no experience in the practice of medicine is not aware of the gross ignorance of even the intelligent laity on subjects medical. Even to some of our most intelligent citizens the chiropractor and the osteopath are as reliable authoritative sources of knowledge as are the professors in our best medical schools. The well meaning talkative doctor is misquoted perhaps oftener than any other type of man. This is especially true when two or more physicians are attending the same patient. Experience has shown that if the patient or relatives are fully cognizant of minor disagreements among the physicians the mental reaction works to the disadvantage of the patient. And when there are real disagreements or when it is a matter of therapeutic inefficiency one will find in the existing principles of medical ethics ample provision for the protection both of the patient and the physician.

### *Modern Aspects of the Diagnosis, Classification and Treatment of Tuberculosis\**

IT IS refreshing to find a writer on tuberculosis who speaks of healing as well as quiescence and arrest. Dr Myers presents an excellent review of the present situation and outlook in tuberculosis from the clinical viewpoint. After a brief concise history of tuberculosis and another chapter on the history of treatment of tuberculosis we find a description of the organism, the method of entrance into the body, diagnosis with tuberculin and a history of the clinical course of the disease. The chapters on diagnosis resume a preliminary acquaintance with the methods of general physical diagnosis and pass at once to the finer details of the recognition of the disease. Fully one half of the volume is devoted to treatment.

We should say that for the general man not the specialist in tuberculosis the volume under review possesses two outstanding values: first for his assistance in the early recognition and diagnosis of tuberculous infection and second for the application of the proper principles of treatment after the patient has returned from his preliminary course of treatment at the sanatorium to remain under the supervision of his own doctor.

### *Pernicious Anemia†*

MILESTONES in our knowledge and understanding of specific diseases are marked by monographic treatises summaries of preexisting knowledge and presentations of recent developments.

The volume on Pernicious Anemia by Beaumont S. Cornell represents such a milestone for while we still know nothing of the etiology of the disease therapeutic progress has taken such a forward step and interest in the disease has been so enhanced, that a monographic review is most timely.

*Modern Aspects of the Diagnosis, Classification and Treatment of Tuberculosis.* By J. Arthur Myers, Associate Professor of Preventive Medicine, Medical and Graduate Schools, University of Minnesota. With an Introduction by David A. Stewart, Associate Professor of Medicine, Manitoba University. Cloth. Illustrated. Pp. 71. The Williams & Wilkins Company, Baltimore, Md. 1927.

†*Pernicious Anemia.* By Beaumont S. Cornell, M.D. (Tor.), Fellow in Duke University. Cloth. 311 pages. Duke University Press, Durham, N. C. 1927.



The author points out how much of our supposed understanding of the disease is pure speculation. At the same time the accumulation of actual facts most of which have not been successfully interarticulated is quite enormous.

The volume is based on a review of 827 contributions to our understanding of the disease and in spite of the enormousness of the literature the author appears not to become lost in a maze of observations but presents a clear discussion of pernicious anemia. This is the first monograph on the disease which has been able to include a comprehensive discussion of the results of liver therapy.

### *Folklore of the Teeth<sup>\*</sup>*

DR KANNER has made a very complete collection of popular superstitions and fancies, ancient, modern, and savage, on the subject of the teeth. Ignorance and superstition while often ludicrous are sometimes dreadful weapons for evil. The savage who destroys his child if the wrong tooth comes in first is pitiable.

We laugh with the author when he tells of the ancient astronomer devoting his life to counting the stars and finding upon questioning that he did not know the number of teeth in his own head. We are interested in the historical bits such as that Louis XIV born with teeth already erupted commenced life by making things disagreeable for the first person with whom he had direct contact, namely his wet nurse. The story of St Apollonia, the Patron Saint of Toothache, is interesting and especially so since the author traces the gradual evolution of the present miraculous story from the original bare facts.

The book is very pleasant reading.

### *Elementary Bacteriology<sup>†</sup>*

IT IS a pleasant surprise to find in an elementary textbook a breaking away from the old traditional methods and the writing of a volume which makes a consecutive story from start to finish and is interesting reading even to one who has had more than the elementary experience with the subject. The first four chapters deal with generalizations, the history of bacteriology, the function of bacteria for good and for evil, and these are followed by chapters presenting in logical sequence morphology, classification, chemistry, food requirements, metabolic activity, influence of environment and interaction of bacteria with their environment, the carbon cycles and nitrogen cycles, nitrogen fixation, the phosphorus and sulphur cycles. Later come chapters on specific applications such as bacteriology of milk food poisoning, bacteria in the arts and industries, bacteria as the cause of disease, immunity, bacteriophage. Then come discussions of specific diseases with a final chapter on filtrable viruses. There is nothing new or startling in the book, in the sense of a new outlook or theory of bacteriology. If there were this would not be a textbook of elementary bacteriology. But the work recommends itself chiefly because it is easy and interesting reading and holds the attention throughout its pages.

### *Microchemistry<sup>‡</sup>*

THIS is the fourth edition since 1921 of this well known little manual of only 200 pages which has already been translated into Russian, Italian, and Spanish. No attempt is made to include all of the methods which have been published or used but only those are included which have been found satisfactory in the author's own laboratory. Methods are given, not only for the usual constituents of urine and blood but also for the common elements important in biochemistry and for oxygen and carbon dioxide in blood, including van Slyke's method for the alkaline reserve.

<sup>\*</sup>Folklore of the Teeth. By Leo Kanner M.D. Yankton State Hospital Yankton South Dakota. Cloth. Illustrated. 316 pages. The Macmillan Company N. Y. 1928.

<sup>†</sup>Elementary Bacteriology. By Joseph E. Greaves M.S. Ph.D. Professor of Bacteriology Utah Agricultural College Logan and Ethelyn O. Greaves M.S. Illustrated. Cloth. Pp. 508. W. B. Saunders Co. 1928.

<sup>‡</sup>Micromethods for the Quantitative Determination of the Constituents of Urine, Blood and Organs in small Quantities for Clinical and Experimental Purposes. By Ludwig Pinckuss. Fourth Edition. Published by Georg Thieme Leipzig.

### *The Advances in Microchemistry\**

THIS is a much larger and more pretentious work than Pineussen's book as it is an attempt to cover all the various fields in which microchemistry has been found useful. Probably most of us have thought of microchemistry in connection with the remarkable analysis which can be made now from a small sample of blood but it has been developed also for use in both the qualitative and quantitative analyses of both organic and inorganic substances. It is remarkable how complete an analysis can be made from a little chip weighing a few milligrams or from a few borings of some precious heirloom or antiquarian relic. The author gives both the general and the specific methods of microchemistry in all of these fields.

### *The Spectrochemical Analysis of Natural Organic Coloring Matters†*

THIS is really a second edition of a book published in 1909 under the title *Chemical Spectroscopy* by the same author. It considers the spectroscope and the spectroscopic methods useful in the study of the respiratory pigments chiefly. Most of the text is given to hemoglobin and all of its various forms and compounds and to its decomposition products including the pigments of the bile and urine. It also considers the closely related chlorophyll, other biologic pigments such as carotin, lutein, urochrome, and indigo and the color producing cholesterol.

### *Die Methodik der Fermente‡*

THIS is a work more or less coordinate with Oppenheimer's well known work on *Ferments* and is designed to contain the practical parts and methods which could not be included in the original work.

Lieferung I contains chapters on polarimetry, refractometry, nephelometry, spectral photometry and colorimetry as well as chapters on viscosity, surface tension, hydrogen ion concentration, electrical conductivity and osmotic pressure.

Lieferung II contains the work on substrates and the methods for obtaining and purifying enzymes.

### Erratum

Through an error the review of 'The Treatment of Chronic Arthritis and Rheumatism' by H. Warren Crowe, the title of which appears on page 387 of the January, 1929, number, will be found on page 481 of the February number under the title "Chronic Rheumatic Diseases Their Diagnosis and Treatment."

The review of "Chronic Rheumatic Diseases Their Diagnosis and Treatment" by Thomson and Gordon, the title of which appears on page 481 of the February number, will be found on page 387 of the January number under the heading 'The Treatment of Chronic Arthritis and Rheumatism.'

*The Advances in Microchemistry* By Gustav Klein and Robert Streblinger. Published by Franz Deuticke, Leipzig and Vienna, 1923.

†*The Spectrochemical Analysis of Natural Organic Coloring Matters* By Prof. Otto Schumm. Pub. by Gustav Fischer, Jena, 1927.

‡*Die Methodik der Fermente (The Methods Used in the Investigation of Ferments)* By Carl Oppenheimer and Ludwig Pincussen. Georg Thieme, Leipzig, 1927.

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Editor WARREN T VAUGHAN, M D

Richmond, Va

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## EDITORIALS

### The Transmission of Leprosy

ALTHOUGH there can be no manner of doubt that leprosy is an infectious disease and that as such it can only be acquired from an antecedent case, nothing accurate is known concerning the method or methods by which the disease is transferred or of how or where lepra bacilli enter the body "

With this succinct statement of an age-long problem Vedder<sup>1</sup> begins a very interesting report of an experimental study in which man was used

While the reports of numerous observers have shown that nasal lesions are common, so common that the respiratory tract at one time was regarded as the common portal of the entry in leprosy, most authorities now believe that this is not so The present opinion rests upon the observations that exclusive leprosy of the nose has never been reported, that positive nasal smears are not encountered in the absence of positive skin lesions, and that the frequency of the first observable lesions on the face together with the free communication with the lymph channels of the nose leads to early nasal infection

The skin, then, appears to be the most common portal of entry of this disease and, in 75 per cent of cases, the skin of an exposed part of the body

This being accepted, we are only on the threshold of the problem and the means whereby invasion occurs have yet to be determined beyond doubt

Leprosy is regarded by almost all authorities as a contagious disease and from time immemorial popular opinion has always assumed contact as an important if not the only avenue of transmission

The discharge of lepra bacilli in enormous numbers from ulcerating lesions and in various excreta and secretions, the fact that leprosy is often a familial disease, and the fact that the density of population influences the incidence of leprosy, all lend support to the importance of contact as a method of transmission

While not denying that contact may be one method of transmission, there is nothing in the evidence as Vedder points out which indicates that it is the only, or even the most important avenue of infection

As opposing evidence are the frequent observations of sharply circumscribed endemic foci, the fact that between infected husbands and wives the uninfected partner contracts the disease in usually only from 5 to 65 per cent, that only about one person in twenty living in close contact with lepers contracts the disease, the freedom from leprosy of doctors, nurses, and attendants, in leprosaria and the consistent failure, except in one disputed case, to inoculate man experimentally

As Hirsch who has exhaustively searched the literature in relation to contact infection says "there is not a single fact that positively and irrefutably shows the transmission of the disease through contagion, on the other hand, there are very cogent facts that contradict such a conception since they are in complete contradiction with all experience concerning the transmission of true contagious diseases"

Most convincing of all, are those cases, several of which have come under Vedder's own observation in which the disease has occurred in Europeans or Americans none of whom were ever in intimate contact and few of whom recall even casual contact with lepers

In view of the evidence thus briefly outlined the possibility that leprosy might be transmitted by some intermediate agency such as the bites of insects very naturally arises and is referred to by numerous investigators Vedder states that there are a number of considerations favoring this conception and quotes from the literature evidence indicating that bedbugs, lice, fleas, ticks, mosquitoes, and skin parasites may be vectors of leprosy

In Vedder's opinion, if leprosy is conveyed by insects, one of the most important should be the mosquito since cases occurring without known previous contact are best explained by a biting, flying insect

He experimented therefore, with *Aedes aegypti*, raised in the laboratory from the eggs

The females thus raised were allowed to bite cases of macular or tubercular leprosy, the skin areas used being first examined microscopically to ensure the presence of numerous bacilli

The mosquitoes were then killed and blood expressed from the abdomen examined. Undoubted lepra bacilli were found in 41 per cent. It appears, therefore, that under proper circumstances leprosy undoubtedly *may* be transmitted by the bite of mosquitoes.

Two human experiments are reported upon by Vedder the subjects being volunteers from the Bilibid prison, long term prisoners conforming to the following conditions

- 1 He must freely volunteer, knowing the nature of the experiment
- 2 He must be a long term prisoner
- 3 He must be a young man in perfect health

4 There must be no leprosy in the family nor any history of contact with lepers, and he must have been in isolation at least two years in Bilibid to exclude the development of leprosy from any other source than the experiment.

Three such volunteers were secured, one being used as a control and inoculated by an intradermal hypodermic injection, the second bitten on three different occasions by a total of 82 freshly infected mosquitoes, and the third bitten by 41 freshly infected mosquitoes allowed to feed repeatedly.

The experiments were conducted in 1927 and one year later none of the prisoners showed any evidence of leprosy, this being in the nature of a preliminary report.

Vedder believes that numerous similar experiments are not only desirable but, in view of the importance of the subject, justifiable.

#### REFERENCES

- 1 Vedder, E. B. A Discussion of the Etiology of Leprosy, With Especial Reference to the Possibility of the Transference of Leprosy by Insects, and the Experimental Inoculation of Three Men, Philippine J. Sc. 37 3, 1928
- 2 Hirsch Handb. der Historisch Geographischen Path. 2 33, 1883

—R. A. K.

### The Etiology of Trachoma

**A**MONG the last studies completed by the late Dr. Hideyo Noguchi, whose untimely death was a severe loss to experimental medicine, there was a series of investigations of trachoma the result of which is published as a complete and well illustrated report.\*

While numerous bacteriologic studies have been made in this disease none, prior to this present investigation had found any microorganism satisfactorily or entirely fulfilling the postulates essential to its recognition as an etiologic agent.

Noguchi's experiments, which are reported in great detail, have resulted in the discovery of a new bacterium (*Bacterium granulosis* N. Sp.), which he believes to be the etiologic agent in trachoma. To support this belief strong confirmatory evidence is produced.

*Bacterium granulosis* is described as a small, motile gram-negative organism, somewhat resembling those of the xerosis group from which it is distinguished, however, by its biologic and chemical characteristics.

\*Noguchi, H. The Etiology of Trachoma. J. Exper. Med. Supplement 2 48 No. 2 1 1928.

When inoculated into chimpanzees, *Macacus* monkeys, and the baboon it produces lesions which grossly and histologically are characteristic of trachoma in the human being. The organism can be found in the lesions so produced, is found in sections from human trachomatous lesions, and is now in its fifth series in animals (culture to rhesus, rhesus to rhesus, rhesus to chimpanzee, chimpanzee to chimpanzee, and chimpanzee to rhesus).

The experimental chronic granular conjunctivitis produced by *Bacterium granulosis* preserved its clinical and histologic characters throughout several passages and the lesions have been shown to be infective as early as seventeen and as late as two hundred and four days after the original culture inoculation.

No other organism obtained from human cases of trachoma produces in animals comparable effects.

It appears therefore, that the etiologic agent of trachoma has at last been isolated.

—B A K

glycemia and a rise in urea and amino acid nitrogen. When synthalin was administered with glycine, there was a marked delay in the return of the amino acids of the blood to the normal level. Blatherwick and his associates, therefore, conclude that synthalin like hydrazene interferes with deamination by causing hepatic injury, that hydrazene causes a more severe injury, and that the hypoglycemia resulting from the use of synthalin may be due to this injury to the liver and a consequent interference with normal glycogenesis. They admit that they are not in a position to know whether or not these results would follow its oral administration in clinical diabetes, since in their experiments on rabbits in which synthalin was given by mouth there was no hypoglycemia nor any increase in the amino acid nitrogen of the blood. In one of these animals they point to a loss of body weight amounting to 0.4 kilo in a period of ten days and a decrease in the urea nitrogen in the blood as an evidence of toxic action. It is recognized that synthalin in too large doses produces a diarrhea and gastric cramps, due probably to the guanidin radicle, and this may have accounted for the loss of weight in this animal, as the dose administered per kilogram of body weight was about 7 mg, while the average dose for a patient is 0.5 to 0.8 mg per kilogram of body weight, and this is discontinued every third or fourth day for one day.

It is generally agreed that synthalin will lower the blood sugar in diabetic patients when given by mouth. Naturally there is great interest as to its effect on liver function, but satisfactory data are lacking on this point. The explanation of Blatherwick and his associates based on interference with glycogenesis due to liver injury is theoretic and not supported by the evidence of Frank and his coworkers. These found in depancreatized dogs that the blood-sugar level was reduced and the urine remained sugar and acetone free after the administration of synthalin by mouth. Such animals are, of course, unable to oxidize carbohydrate given as such. Lublin<sup>8</sup> has shown the effect on the respiratory quotient of the administration of glucose with and without synthalin. In the same patient, a severe diabetic, 30 gm of glucose administered by mouth was not oxidized, the R Q remaining between 0.65 and 0.708. Following a day on which 40 mg of synthalin were administered by mouth the experiment was repeated and the R Q was never below 0.721 and rose to 0.832. This is most significant, and points to the utilization of carbohydrate by a patient who was otherwise unable to make any use of it. The observations were repeated on several other patients with similar results, the R Q in one case rising to 0.967. These results cannot be explained on the basis of interference with glycogenesis.

A total of twelve cases have been under our observation, the longest, since July, 1927. The summary of the cases and the effect of synthalin are given. We have never felt justified in using the drug unless the patient could not remain sugar free and maintain a moderate blood-sugar level on a calorie diet compatible with the demands of her daily life. The patients usually did better while in the hospital, probably due to the regulation of the diet, which remains an important factor in the treatment of diabetes.

## REPORT OF CASES

CASE 1—M H, female aged forty five weight 162½ pounds height 67 inches, admitted originally to the hospital April 2 1927 History of chronic diabetes On admission the patient was drowsy, mentally confused and with a strong acetone odor to her breath Blood sugar 370 mg This patient was first treated with insulin and was discharged on April 21, on a total of 50 units daily in two divided doses and a diet of 2100 cal The insulin dosage

TABLE I  
CASE 1

DATE	URINE		BLOOD SUGAR	INSULIN UNITS	SYNTHALIN MG	NO OF DAYS	DIET
	SUGAR	ACETONE					
Sept 0	0	0	120	8	10	Daily	C - 100
Sept 10	0	0			10		P - 60
Sept 11	0	0		2	10		F - 150
Sept 12	0	0		0	20		Cal - 2100
Sept 13	0	0		0	20		The diet was
Sept 15	0	0	152	0	30		not changed
Sept 29	0	0	109	0	20		at any time
Oct 13	0	0	135	0	alternating with 30		
Oct 27	0	0	142	0	30	For 2 days off 1 day	
Nov 2	0	0	*177	0	30	For 3 days off 1 day	
Nov 17	Tr	0	154	0	30	Same	
Dec 8	0	0	145	0	30	Same	
1928							
Jan 9	0	0	92	0	30	For 2 days off 1 day	
Feb 16	0	0	100	0	30	Same	
Mar 1	0	0	118	0	20	q 2 d	
Mar 22	0	0	130	0	20		
Apr 10	0	0	138	0	20		

The patient had been constipated for several days and had had a cold

TABLE II  
CASE 2

DATE 1927	URINE		BLOOD SUGAR	SYNTHALIN MG	NO OF DAYS	C	P	F
	SUGAR	ACETONE						
July 5	Tr	Tr	120	0		40	40	100
July 15	Tr	0	-	20		60	60	150
July 16	0	0	-	20			Same	
July 17	0	0	-	50			Same	
July 18	-	-	-	0			Same	
July 19	0	0	102	25	Daily		Same	
July 21	0	0	-	10	Daily		Same	
July 28	0	0	104	10	Daily		Same	
Aug 9	0	0	138	12½	Daily		Same	
Aug 30	0	0	130	10	Daily		Same	
Sept 7	0	0	85	10	Daily		Same	
Sept 20	0	0	87	10	Daily		Same	
Oct 13	0	0	131	10	Daily		Same	
Nov 10	0	0	107	10	Daily		Same	
Dec 15	0	0	110	10	Daily		Same	
1928								
Jan 5	0	0	109	10	Daily		Same	
Feb 16	0	0	123	10	On 2 days off 1 day			
Mar 10	0	0	163	0	Discontinued until April 5	60	60	120
Apr 5	+	0	180	10	Begun again daily		Same	
May 10	0	0	125	10	On 3 days off 1 day		Same	



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## News and Notes

The next annual meeting of the American Society of Clinical Pathologists will be held in Portland, Oregon, July 5, 6, and 8, 1929. All members are urged to make plans to attend this convention which besides promising a great intellectual feast will also enable them to visit our great far west.

The attention of the members of the American Society of Clinical Pathologists is called to the fact that at our next convention in Portland, Oregon, one half day will be devoted to a group of presentation and general discussion of the subject of "Undulant Fever." Participation in this symposium by all the members is earnestly requested by the Program Committee and Research Committee.

Members will kindly report any change in address to the Secretary of the American Society of Clinical Pathologists, Metropolitan Building, Denver, Colorado.

Members who have not made reservations for the next convention should do so immediately by writing direct to Dr H H Foscett, Medical Arts Building, Portland, Oregon. In view of the crowded condition of the Portland Hotel, our headquarters, during the American Medical Association Convention, members who have already made reservations for both the A S and the members may be asked to move to another hostelry during the A M Convention. Reservations in other hotels, however, are ample and Dr Foscett will be glad to make personal arrangements.

# *The Journal of Laboratory and Clinical Medicine*

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No 8

## *CLINICAL AND EXPERIMENTAL*

### *SYNTHALIN IN THE TREATMENT OF DIABETES\**

By ELAINE P. RALLI M.D. AND CONNIE M. GUION M.D., NEW YORK

IN VIEW of the widespread interest in synthalin and the scarcity of clinical reports following its use the cases in our clinic at present under treatment are presented. In Frank Nothmann and Wagner's original articles<sup>1 1 1b</sup> they state that synthalin is a derivative of aminopenthylen guanidin in which, by lengthening the methyl chain the toxicity of the parent substance is greatly lessened. Their results experimental and clinical showed that synthalin caused a reduction of blood sugar in both the normal and the diabetic but just how this oxidation of carbohydrate was effected was not explained. Other investigators who obtained similar results clinically are Joslin, Carasco,<sup>3</sup> Duncan,<sup>4</sup> and Ringer and his associates.

Lewis and Izume<sup>5</sup> showed that hydrazene produces a hypoglycemia and that this is accompanied by an increase in the amino acids in the blood due to a failure of deamination resulting from liver injury. Their explanation of the hypoglycemia is that the supply of glucose available for combustion is decreased because glycogenesis, the transformation of noncarbohydrate material to glucose, is interfered with due to the hepatic injury produced by hydrazene. This work suggested to Blatherwick and his associates<sup>7</sup> that synthalin might produce its effect in a similar manner. They administered synthalin by stomach, subcutaneously and intravenously to rabbits. The doses in each case were much larger per kilogram of body weight than are used therapeutically, and it must be remembered that the subcutaneous and intravenous routes are never used clinically. In the rabbits receiving synthalin by stomach there was no effect either on the sugar or the amino acid content of the blood. The latter is taken as an index of hepatic injury. On the other hand, subcutaneous and intravenous administration produced a hypo

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glycemia and a rise in urea and amino acid nitrogen. When synthalin was administered with glycine, there was a marked delay in the return of the amino acids of the blood to the normal level. Blatherwick and his associates, therefore, conclude that synthalin like hydiazene interferes with deamination by causing hepatic injury, that hydiazene causes a more severe injury, and that the hypoglycemia resulting from the use of synthalin may be due to this injury to the liver and a consequent interference with normal glyconeogenesis. They admit that they are not in a position to know whether or not these results would follow its oral administration in clinical diabetes, since in their experiments on rabbits in which synthalin was given by mouth there was no hypoglycemia nor any increase in the amino acid nitrogen of the blood. In one of these animals they point to a loss of body weight amounting to 0.4 kilo in a period of ten days and a decrease in the urea nitrogen in the blood as an evidence of toxic action. It is recognized that synthalin in too large doses produces a diarrhea and gastric cramps, due probably to the guanidin radicle, and this may have accounted for the loss of weight in this animal, as the dose administered per kilogram of body weight was about 7 mg, while the average dose for a patient is 0.5 to 0.8 mg per kilogram of body weight, and this is discontinued every third or fourth day for one day.

It is generally agreed that synthalin will lower the blood sugar in diabetic patients when given by mouth. Naturally there is great interest as to its effect on liver function, but satisfactory data are lacking on this point. The explanation of Blatherwick and his associates based on interference with glyconeogenesis due to liver injury is theoretic and not supported by the evidence of Frank and his coworkers. These found in depancreatized dogs that the blood-sugar level was reduced and the urine remained sugar and acetone free after the administration of synthalin by mouth. Such animals are, of course, unable to oxidize carbohydrate given as such. Lublin<sup>8</sup> has shown the effect on the respiratory quotient of the administration of glucose with and without synthalin. In the same patient, a severe diabetic, 30 gm of glucose administered by mouth was not oxidized, the R Q remaining between 0.65 and 0.708. Following a day on which 40 mg of synthalin were administered by mouth the experiment was repeated and the R Q was never below 0.721 and rose to 0.832. This is most significant, and points to the utilization of carbohydrate by a patient who was otherwise unable to make any use of it. The observations were repeated on several other patients with similar results, the R Q in one case rising to 0.967. These results cannot be explained on the basis of interference with glyconeogenesis.

A total of twelve cases have been under our observation, the longest, since July, 1927. The summary of the cases and the effect of synthalin are given. We have never felt justified in using the drug unless the patient could not remain sugar free and maintain a moderate blood-sugar level on a caloric diet compatible with the demands of her daily life. The patients usually did better while in the hospital, probably due to the regulation of the diet, which remains an important factor in the treatment of diabetes.

## REPORT OF CASES

CASE 1—M H, female aged forty five weight 162½ pounds height 67 inches, admitted originally to the hospital April 2, 1927 History of chronic diabetes On admission the patient was drowsy, mentally confused and with a strong acetone odor to her breath Blood sugar 370 mg This patient was first treated with insulin and was discharged on April 21, on a total of 50 units daily in two divided doses and a diet of 2100 cal The insulin dosage

TABLE I  
CASE 1

DATE	URINE SUGAR ACETONE		BLOOD SUGAR	INSULIN UNITS	SYNTHALIN MG	NO OF DAYS	DIET
Sept 9	0	0	120	8	10	Daily	C - 100
Sept 10	0	0		5	10		P - 60
Sept 11	0	0		2	10		F - 150
Sept 12	0	0		0	20		Cal - 2100
Sept 13	0	0		0	20		The diet was
Sept 15	0	0	152	0	30		not changed
Sept 29	0	0	109	0	20		at any time
Oct 13	0	0	135	0	20 alternating with 30		
Oct 27	0	0	142	0	30	For 2 days off 1 day	
Nov 2	0	0	177	0	30	For 3 days off 1 day	
Nov 17	Tr	0	154	0	30	Same	
Dec 8	0	0	145	0	30	Same	
1928							
Jan 9	0	0	92	0	30	For 2 days off 1 day	
Feb 16	0	0	100	0	30	Same	
Mar 1	0	0	118	0	20	q 2 d	
Mar 22	0	0	130	0	20		
Apr 19	0	0	138	0	20		

The patient had been constipated for several days and had had a cold

TABLE II  
CASE 2

DATE 1927	URINE SUGAR ACETONE		BLOOD SUGAR	SYNTHALIN MG	NO OF DAYS	C	P	F
July 5	Tr	Tr	100	0		40	40	100
July 15	Tr	0	-	20		60	60	150
July 16	0	0	-	25			Same	
July 17	0	0	-	50			Same	
July 18	-	-	-	0			Same	
July 19	0	0	102	25	Daily		Same	
July 21	0	0	-	10	Daily		Same	
July 28	0	0	104	10	Daily		Same	
Aug 9	0	0	138	12½	Daily		Same	
Aug 30	0	0	130	10	Daily		Same	
Sept 7	0	0	85	10	Daily		Same	
Sept. 20	0	0	87	10	Daily		Same	
Oct 13	0	0	131	10	Daily		Same	
Nov 10	0	0	107	10	Daily		Same	
Dec 15	0	0	110	10	Daily		Same	
1928								
Jan 5	0	0	109	10	Daily		Same	
Feb 16	0	0	123	10	On 2 days off 1 day			
Mar 19	0	0	163	0	Discontinued until April 5	60	60	120
Apr 5	+	0	180	10	Begun again daily		Same	
May 10	0	0	120	10	On 3 days off 1 day		Same	

was reduced the second of May to 39 units daily, and on July 7, to 30 units. On the ninth of September, at which time she was receiving a daily dose of 12 units of insulin, 1 of the insulin units were replaced with 10 mg of synthalin. On September 10, the insulin dose was decreased to 5 units, the synthalin remaining at 10 mg. On the eleventh of September the insulin was reduced to 2 units synthalin still 10 mg, and on the next day insulin was discontinued and two 10 mg tablets of synthalin were given. The urine during this period remained sugar free. The diet was unchanged.

CASE 2—R R female, aged forty two, weight 190 pounds, height 60 inches. Associated symptoms pruritus vulvae. This patient was sensitive to insulin. She had been admitted to the hospital for treatment on two separate occasions. The first time was in October, 1926, with a blood sugar of 214 mg, and sugar and acetone in her urine. After six weeks she was discharged on a diet of 1525 calories and 10 units of insulin daily. Her second admission was in January, 1927. Her complaint was a rash following insulin injections. This consisted of a maculopapular eruption on both arms, across the chest and back, and on her thighs. The left arm and hand were considerably swollen, and there was a good deal

TABLE III

CASE 3

DATE		URINE		BLOOD SUGAR		BLOOD SUGAR		NO OF DAYS		DIET		
1927		SUGAR	ACETONE	SUGAR	MG					C	P	F
Oct	18	++	+		0					Thrice cooked 50 per cent vegetables only were allowed in this period		
Oct	19	0	0	273	0							
Oct	20	22	+		0							
Oct	21	0	+		0							
Oct	22	0	+	200	0							
Oct	23	0	0		0							
Oct	24	0	0		0							
Oct	25	0	+		0					50 75 110		
Oct	26	0	+		20							
Oct	27	0	+		30							
Oct	28	0	+	159	45							
Oct	29	0	+		5							
Oct	30	Tr	+		35							
Oct	31	Tr	0	172	5							
Nov	1	0	0		25					Same		
Nov	2	+	0							Same		
Nov	3	+	0		45					Same		
Nov	4	0	0		45					Same		
Nov	5	0	0							Same		
Nov	6	0	0		45					Same		
Nov	7	0	0	153	45					Same		
Discharged from hospital and treated in clinic												
Nov	17	0	0	222	35	On 3 days, off 1 day				Same		
Dec	1	++	Tr	215	55	On 3 days, off 1 day				Same		
Dec	8	++	Tr	185	45	On 3 days, off 1 day				Same		
Dec	15	++	Tr	179	45	On 2 days, off 1 day				Same		
Dec	22	++	Tr	212	45	On 1 days, off 1 day		60		60	110	
1928												
Jan	5	++	Tr	127	25	On 2 days, off 1 day				Same		
Jan	19	Tr	0	227	35	On 3 days, off 1 day				Same		
Jan	26	0	0	190	45	On 3 days, off 1 day				Same		
Feb	2	0	0	105	25	On 3 days, off 1 day				Same		
Feb	9	0	0	177	45	On 2 days, off 1 day				Same		
Feb	16	Tr	0	202	45	On 3 days, off 1 day				Same		
Mar	15	Tr	0	215	45	On 3 days, off 1 day				Same		
Mar	29	Tr	0	168	45	On 3 days, off 1 day				Same		
Apr	12	0	0	168	45	On 3 days, off 1 day				Same		
Apr	26	Tr	0	181	45	On 3 days, off 1 day				Same		
May	10	Tr	0	194	45	On 3 days off 1 day				Same		

of itching. Her blood sugar was 103 mg, which rose to 136 mg after breakfast. Skin tests showed the patient sensitive to insulin. She was discharged on a diet of 1000 calories and a blood sugar of 77 mg. Her weight at this time was 156 pounds. After discharge the patient was followed in the clinic. Her blood sugar varied from 120 mg to 150 mg and there were traces of sugar in her urine. On July 5 the patient was prepared for synthalin treatment the diet being increased to 1220 calories, consisting of C 40 P 40 F 100. The following chart will show her progress and includes the period when the drug was stopped for a period of time at which time her blood sugar rose and sugar reappeared in the urine.

CASE 3—F. M. female aged fifty seven weight 181½ pounds height 60 inches. History of chronic diabetes. Admitted to the hospital for treatment of increasing weakness and pains in the legs.

TABLE IV

(CASE 3)

DATE	1927	URINE		BLOOD SUGAR		DIET			BLOOD PRESSURE
		SUGAR	ACETONE	SUGAR	MG	C	P	F	
Jul	26	+	Tr	200	0	40	40	100	190/100
Jul	29	+	Tr		0	The diet remained unchanged			
Jul	30	+	Tr		0				
Jul	31	+	Tr		0				
Aug	1	+	Tr	200	0				130/80
Aug	2	+	Tr		0				135/80
Aug	3	+	Tr		0				135/80
Aug	4	+	Tr		0				130/80
Aug	5	+	Tr		0				135/80
Aug	6	+	Tr		0				135/80
Aug	7	+	Tr		0				130/80
Aug	8	+	Tr		20				132/80
Aug	9	0	0	108	20				130/60
Aug	10	0	0		20				120/80
Aug	11	0	0		20				140/72
Aug	12	0	0	174	20				145/00
Aug	13	0	0		20				128/70
Aug	14	0	0		20				130/78
Aug	15	0	0	161	20				142/70
Aug	16	0	0		20				130/70
Aug	17	Tr	Tr		20				142/78
Aug	18	0	0		25				130/70
Aug	19	0	0		20				134/74
Aug	20	0	0		20				120/60
Aug	21	0	0		20				120/70
Aug	22	0	0	170	20				120/80
Aug	23	0	0		20				120/80
Aug	24	0	0	110	20				110/60
Aug	25	0	0		20				110/60
Aug	26	0	0	147	20				110/60
Sept	27	0	0	98	20				130/80
Sept	28	0	0	10	20				116/70
Oct	11	0	0	134	25				148/90
Oct	20	0	0		20				110/70
Oct	21	0	0	113	20				
Nov	10	0	0		20				
Nov	17	0	0	121	20				
Dec	1	0	0	178	20				100/100
Dec	8	0	0	90	20				
1928									
Feb	16	0	0	14		None since Dec 1 Cardiac condition poor Patient in failure Entered hospital			100/100
Mar	22	0		100					

TABLE IV  
CASE 4—CONTINUED

DATE 1928	URINE		BLOOD SYNTHALIN		INSULIN (UNITS)	DIET			BLOOD PRESSURE
	SUGAR	ACETONE	SUGAR	MG		C	P	F	
Mar 23	0	+	197	0	8	100	50	70	148/ 90
Mar 24	0	+		0	8		Same		
Mar 25	0	0		0	8		Same		
Mar 26	0	0	190	0	10		Same		
Mar 27	0	0		0	10		Same		138/ 76
Mar 28	0	0	178	0	10		Same		
Mar 29	0	0		0	10		Same		
Mar 30	0	0	178	0	10		Same		
Apr 1	0	0		0	0	52	32	66	
Apr 2	0	0	178	0	0		Same		
Apr 3	0	0		0	0		Same		128 /70
Apr 4	0	0	195	0	5		Same		
Apr 5	0	0		0	5		Same		
Apr 6	0	0	170	0	5		Same		
Apr 7	0	0		0	5		Same		
Apr 8	0	9		0	5		Same		
Apr 9	0	0	178	0	5		Same		
Apr 10	0	0		25	0	40	40	80	
Apr 11	0	0	139	25	0		Same		
Apr 12	0	0		25	0		Same		
Apr 13	0	0	130	25	0		Same		120/ 70
Apr 14	0	0		25	0		Same		
Apr 15	0	0		0	0		Same		
Apr 16	0	0		25	0		Same		
May 3	0	0	138	25	Daily 0	50	50	100	130/ 80

CASE 4—M W, female, aged fifty one, weight 168½ pounds, height 56 inches Diabetes since 1925 This patient has auricular fibrillation and a tendency to hypertension which is especially marked when she has a high blood sugar She was admitted to the hospital on July 28, 1927, with signs of cardiac failure, pruritis and a blood pressure of 190/100 She was digitalized, and has been on digitals ever since

TABLE V  
CASE 5

DATE	URINE		BLOOD SYNTHALIN		NO OF DAYS	DIET			CALORIES
	SUGAR	ACETONE	SUGAR	MG		C	P	F	
Sept 22	+++	+++		0		5% vegetables			600
Sept 29	+++	+	308	30	Daily	33	33	68	900
Oct 4	0	0	130	10	Daily	33	33	68	900
Oct 14	0	0	134	0	None	35	35	80	1024
Oct 27	0	0	171	10	Daily	35	35	80	1024
Nov 3	0	0	185	20	Daily	35	35	80	1024
Nov 10	0	0	158	20	On three days, off one day	35	35	80	1024
Nov 17	0	0	135	20	On two days, off one day	40	40	100	1220
Dec 1	0	0	116	10	On three days, off one day	50	50	125	1525
Dec 15	0	0	123	10	On three days, off one day	50	50	125	1525
Dec 29	0	0	94	10	On three days, off one day	50	50	125	1525
Jan 8									
Jan 19	0	0	100	None	since Jan 5	50	50	125	1525
Jan 28	0	0	120	0		50	50	125	1525
Mar 1	0	0	148	0		50	50	125	1525
Mar 22	0	0	165	0		50	50	125	1525
May 17	0	0	206	0		Has not adhered to diet			

CASE —B K, female aged forty eight, weight 143 pounds height 61 inches Has known she had diabetes for about one year Lateh has felt dizzy and weak Pruritis vulvae severe headache, thirst and polyuria On admission to the clinic on Sept 22 the patient appeared dopey, slight odor of acetone on the breath and tongue reddened The patient was advised to enter the hospital but refused She was put on a diet consisting of 5 per cent vegetables and two oranges daily and sent in for a blood sugar

TABLE VI

## CASE 6

DATE	URINE			BLOOD SYNTHALIN		NO OF DAYS	DIET		
	DIACETIC	ACETONE	SUGAR	GLUCOSE	MG		C	P	F CALORIES
1927									
Dec 20	+	+	++		0		66	24	37 693
Dec 21	+	+	++	225	0		66	24	37 693
Dec 22	+	+	06	154	0		66	24	37 693
Dec 23	+	+	04		0		66	24	37 693
Dec 24	+	+	04		0		66	24	37 693
Dec 25	+	0	0		0		75	25	35 715
Dec 26	0	0	0		0		75	25	35 715
Dec 27	0	0	03	155	30	Daily	75	25	35 715
Dec 28	0	0	0		30	Daily	101	35	43 931
Dec 29	0	0	0		50	Daily	101	35	43 931
Dec 30	0	0	0	150	0	None	101	41	48 1000
Clinic Record									
1928									
Jan. 5	0	0	Tr		30	For 3 days	100	45	60
Jan. 12	0	0	+	130	30	For 3 days	100	45	80
Jan 19	0	0	+	125	30	For 3 days	100	45	80
Jan 26	0	0	+	111	30	For 3 days	100	45	80
Jan 31	0	0	0	150	30	For 2 days	100	45	80
Feb 10	0	0	0	171	30	For 2 days	100	45	80

TABLE VII

## CASE 7

DATE	URINE			BLOOD SUGAR	INSULIN UNITS	SYNTHALIN		DIET		CALORIES
	DIACETIC	ACETONE	SUGAR			MG	C	P	F	
1928										
Oct 25	0	+	+++			0	65	40	80	1140
Oct 26	0	+	++			0		Same		
Oct 27	0	+	+	150	10	0		Same		
Oct 28	0	+	+		10	0		Same		
Oct 29	0	+	+		20	0		Same		
Oct 30	+	+	Tr	166	10	0		Same		
Oct 31	0	0	Tr		10	0		Same		
Nov 1	0	0	Tr		10	0		Same		
Nov 2	0	+	Tr		10	0		Same		
Nov 3	0	+	Tr		10	0	75	45	90	1290
Nov 4	0	+	0		20	0		Same		
Nov 5	0	+	0	217	15	0				
Nov 6	0	+	0		40	0				
Nov 7	0	0	Tr	168	20	0				
Nov 8	0	0	Tr		20	0	75	50	110	
Nov 9	0	+	Tr		25	0		Same		
Nov 10	0	0	0	250	25	0		Same		
Nov 11	0	0	0		40	0		Same		
Nov 12	0	0	Tr	194	10	10		Same		
Nov 13	0	0	0		20	10		Same		
Nov 14	0	0	0	154	10	20		Same		
Nov 15	+	+	0		10	20		Same		
Nov 16	+	0	0	195	10	20		Same		
Nov 17	+	0	0		5	30		Same		



younger diabetics We have used it in cases which we could not keep sugar free on diet alone and who were unwilling to take insulin

#### REFERENCES

- <sup>1</sup>Frank, Nothmann, and Wigner *Klin Wchnschr*, 1926, xlv
- <sup>1a</sup>Frank, Nothmann, and Wigner *Deutsch med Wchnschr*, 1926, xlix, 50
- <sup>1b</sup>Frank *Verhandl d Gesellsch f Ver u Stoff wechsel*, 1927
- <sup>2</sup>Joshn, E P *Jour Clin Investigation*, 1927, iv, 435
- <sup>3</sup>Carraseo *Rev med de Barcelona*, March, 1927, vii, 246
- <sup>4</sup>Duncan *Jour Am Med Sc*, February, 1928, clxxv, 196
- <sup>5</sup>Ringer, Biloon, Harris, and Landy *Arch Int Med*, April, 1928, xli, 453
- <sup>6</sup>Lewis and Izume *Jour Biol Chem*, 1926 1927, lxxi, 33
- <sup>6a</sup>Izume and Lewis *Jour Biol Chem*, 1926 1927, lxxi, 51
- <sup>7</sup>Blatherwick, Sghyun, and Hill *Jour Biol Chem* 1927, lxxv, No 3
- <sup>8</sup>Lablin *Arch f Exper Path and Pharmacol*, 124 Band, July, 1927, 118

### ANAPHYLAXIS IN HEMOPHILIA\*

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HEMOPHILIA has been the subject of much research with respect to its etiology and treatment, and it is not unnatural that many theories should be evolved toward explaining its nature. The disease is as old as recorded history itself, but seems still to defy the most energetic methods directed toward the investigation of its cause. In 1921, Vines<sup>1</sup> accidentally noticed a marked reduction in the clotting time of the blood in a hemophiliac who was receiving serum subcutaneously as a form of treatment. In following this case he was impressed with the possibility of controlling the bleeding by induced anaphylaxis, and suggested its trial more as a field for further research than as a specific cure of the disease.

Vines has reported his results with the use of anaphylaxis in three cases of true hemophilia. The method employed consisted of injecting 4 c.c. of normal horse serum subcutaneously, and then in eight to fourteen days repeating the injections with a much smaller amount (0.2 c.c.), and noting the presence of a wheal. In analyzing these results one is impressed by the fact that the bleeding time in each case varied considerably, and that bleeding episodes continued to occur during the treatment. The first case in which this method was carried out was followed over a period of four and one-half months during which time the patient remained sensitive to horse or sheep serum as shown by intradermal tests. In this case it was noted that there was a reduction in the clotting time immediately after the intradermal injection of serum but that bleeding episodes occurred following the injection, regardless of the degree of sensitization. However, Vines considers that a clinical cure of hemophilia had been effected over a period of twenty-nine days, in spite of the appearance of subcutaneous ecchymoses, which he suggests might be due to "an increased fragility of the vascular walls."

The second case which Vines studied was followed over a period of

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four months. In this case he noticed that the clotting time was quite low immediately following a hemorrhage but varied considerably during the time the patient was sensitive to serum. The prothrombin quotients ( $\frac{\text{prothrombin time of patient}}{\text{prothrombin time of control}}$ ) varied in this case between 0.7 and 2.4, but he believed that the clotting time remained normal for more than forty days, and there were no bleeding episodes noted during this time. In this case, then, there is a remarkable retrogression of hemophilic accidents over a period of four months, during which the clotting time of the blood was estimated at weekly intervals.

The third case was followed for one month during which the prothrombin quotient varied between 2.5 and 8.0. In this case four intradermal injections were given after the preliminary dose of 7 cc. of normal sheep serum. The coagulation ratio varied between 1.2 and 8.0 and the results were further confused by the necessity of transfusing the father's blood to the patient. Vines considers the case as unsuccessful although he states that immediately after each injection there was an acceleration of the coagulation rate.

A differentiation seemingly must be made among the possible effects from the serum itself, the induced anaphylactic state and the reactions which must have occurred due to the injection of foreign proteins. In commenting on these objections, Vines quotes abundant evidence from other workers which would make it appear that usually simple serum injections are not satisfactory in the treatment of hemophilia, and that the danger of anaphylactic shock would be imminent in certain cases. He further quotes Sahli on the point that immediately after, and for a period following hemophilic hemorrhages, the blood coagulation may be shorter than normal.

The problem was then studied by C. A. Mills who used the technique suggested by Vines. He reports that bleeding in hemophiliacs can be controlled at will by anaphylaxis. Mills studied four cases and in three of these data concerning the treatment are given. In one case in which, apparently, the method of Vines was carried out the clotting time was reduced from ten minutes to four and one half minutes. In another case the clotting time was five minutes before a transfusion of blood from a patient sensitive to horse serum. Later 4 minims of horse serum were given and eight minutes after this dose the clotting time had been reduced from ten minutes to two and one half minutes. In the final case reported by Mills on September 25 the clotting time was five minutes. Then, forty five minutes after an intracutaneous dose of horse serum (4 minims) the clotting time was three and one half minutes. On October 4, it was four minutes and one hour after the administration of serum (4 minims) the clotting time was reduced to two minutes and forty seconds. Commenting on this case Mills says: "We again see the corrective influence of the local protein reaction on the hemophilic condition."

In these reports there seems to be lacking data necessary to establish any therapeutic value of anaphylaxis over a period of time sufficient to justify the procedure. The results of Mills are of the same nature as those of Vines in that the immediate effect of the serum injection was that of reducing the clotting time, but in no case is there evidence presented to show that during the period of reaction itself, was the clotting time influenced in any way.

Vines states that the duration of the effect of the treatment is dependent both on duration of the anaphylaxis and upon the severity of the hemophylic condition. In this respect it is possible that in the case here reported the clotting time was too high to expect a satisfactory reduction by means of the method advocated. However, Mills says "This (procedure) is of very great value clinically, since we may now keep our hemophiliacs sensitized to some protein." And later he reports<sup>3</sup> "good success in five hemophiliacs by making them sensitive to sheep or hen serum," and that a study of their blood before and after the treatment shows that the fundamental fault has been corrected.

In order to test this hypothesis, it was decided to apply the procedure to a case of hemophilia, and observe the results over a sufficiently long period, to test its clinical value.

A. F. D., a white boy, aged twenty, entered the University Hospital on March 9, 1927, complaining of bleeding and painful, swollen joints.

The family history through four generations is charted. The patient's mother has been married twice. A half brother of the patient died at five years from hemorrhage after having the frenulum linguae taken out by the family physician. One cousin also died at two years of age of hemorrhage.

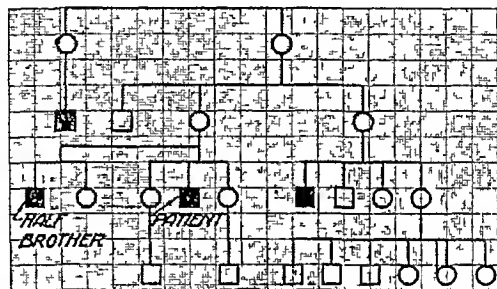


Fig 1—Family chart. Circles represent females. Black squares represent hemophiliacs. It will be seen that the Law of Nasse—that the disease is transmitted by the unaffected female—is fulfilled and that the patient is a true hemophiliac.

At the age of six months the patient's mother observed dark spots on his body which were transient. At about five years of age he had swollen ankles with the overlying skin turning black and clearing, later, the knees and elbows became afflicted as well. Epistaxis was frequent in infancy but the joint symptoms were most prominent. At fifteen years of age he had hematuria over a period of three weeks. The bleeding episodes occurred at irregular intervals and during the past few years they apparently became less frequent.

The following data give a complete summary of the case through one year in chronological order.

Admitted March 9, 1927. Bleeding time ten minutes, clotting time ninety minutes (Howell method). Urine clear. Hemoglobin 75 per cent, platelets 250,000. Blood type 4 (Jansky). The left leg was put in a cast in an attempt to correct a fixed equinus deformity of the left ankle.

March 25, correction being slowly obtained.

March 31, Cast removed and adhesive traction applied to obtain dorsiflexion of foot.

April 5, small hemorrhage into left ankle. Orthopedic treatment discontinued.

April 21, admitted to ward for nonspecific protein therapy.

April 22, patient given 500,000,000 killed typhoid bacilli intravenously at 4:30 P.M.

The white blood counts, during this experiment, varied between 7,900 and 16,600, quite parallel to the temperature curve. Tables I to III show the

**THE**

[illegible]

TABLE II

DATE	CLOTTING TIME	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
4/26/27	100 min	85 min	94
4/30/27	100 min	56 min	62
Pain in left leg and knee	95 min	55 min	61
5/3/27			
Pain in right knee	100 min	60 min	66
5/8/27			
Right knee very sore	95 min	53 min	59
5/10/27			
5/12/27	100 min	60 min	66
5/24/27	110 min	65 min	71
X radiation over spleen			
5/25/27			
6/3/27			
X radiation over spleen			

TABLE III  
HIGH PURINE DIET

DATE	CLOTTING TIME	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
6/7/27	102 min	60 min	66
6/22/27	95 min	56 min	61
6/27/27	97 min	58 min	64
6/29/27	95 min	56 min	61
7/2/27	24 min	20 min	22
7/7/27	107 min	96 min	106
7/11/27	95 min	60 min	66
Pain and swelling of left arm	Ice bag to elbow		
7/13/27	100 min	58 min	64
Left arm very sore, unable to feed self			
7/14/27			
Few RBC in urine	Positive guaiac on stool		
7/16/27			
Hemoglobin 80 %	RBC 3,900,000	Eosinophils 1%	
7/20/27	97 min	60 min	66
8/3/27	105 min	90 min	100
8/6/27	110 min	85 min	94
8/10/27	Patient discharged		

TABLE IV

	CLOTTING TIME	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
12/9/27	101	74	82
12/10/27	Intradermal test with normal horse serum tried with negative results 40 cc normal horse serum (PD) given subcutaneous		
12/17/27	Wrists and hands swollen		
12/19/27	72	54	60
12/20/27	0.2 cc normal horse serum (PD) injected intradermally. Marked reaction with development of large wheal and extensive hyperemia. Patient developed an urticaria 7 days after this injection.		
12/21/27	74	59	66
1/13/28	78	62	69
1/20/28	0.2 cc normal horse serum (PD) injected intradermally. Marked reaction with development of wheal and hyperemia.		
1/24/28	105	80	88
1/25/28	105	85	89
1/26/28	101	79	87
1/27/28	Blood in stool		
1/28/28			

changes which occurred. The data differ in no way from that usually obtained in normal individuals who receive intravenous protein except that the actual clotting time of the blood varied within wider limits.

This experiment occupied one month the injections being given on April 23, April 30 and May 10. Between the second and third injections the patient developed a hemorrhage into the right knee associated with considerable swelling and pain. It seems clear then that the effect of shock therapy on this patient differs only in degree from that seen in experimental animals, and that the reduction of the clotting time is a temporary, rather than a permanent phenomenon.

The patient was discharged August 10, 1927, and he returned to the clinic December 8, 1927, for further observation with regard to the effect of anaphylaxis on the clotting time. Table IV gives in detail the results of the experiment. It is obvious that no favorable change occurred in the patient's condition while under observation. There were on the contrary continuous manifestations of hemophilia such as hematuria and melena as well as joint and muscle hemorrhages.

#### COMMENT

From the results in this case we are scarcely justified in assuming that anaphylaxis or shock therapy has a place in the treatment of hemophilia, and from the published results of Mills there is no indication that a lasting benefit was secured by maintaining an anaphylactic state toward foreign serum. Our results with protein shock therapy indicate that a considerable reduction in the clotting time may be secured *while fever is present*.

In considering the results of both Vines and Mills it must not be forgotten that, in many cases of hemophilia there is considerable variation in the clotting time of the blood. For example in three cases reported by Davidson and McQuarrie<sup>4</sup> the variations were as follows:

CASE	LOW MINUTES	HIGH MINUTES
R R	33	70
E H T	36	73
C J	70	387

In our own case the clotting time varied between twenty four and one hundred and five minutes when the method of Howell<sup>5</sup> was employed. It will be noted in the tables that bleeding episodes were taking place at intervals regardless of the treatment employed.

#### SUMMARY

A case of hemophilia is reported in which an attempt was made to modify permanently the clotting time of the blood by anaphylaxis.

The method suggested by Vines (1921) was used but no permanent influence of the treatment could be detected in this case and the favorable results reported by Mills are not confirmed.

#### REFERENCES

- 1 Vines H W C. Quart J Med 13 257 1920
- 2 Mills, C A. Am J Physiol 76 632 1926
- 3 Mills, C A. Am J Med Sc 172 71, 1926
- 4 McQuarrie E C, and Davidson I. Johns Hopkins Hosp Bull 36 343, 1925
- 5 Howell W H. Arch Int Med 13 76 1914

# THE EFFECT OF CALCIUM CHLORIDE UPON THE TOXICITY OF BILE\*

By WILLIAM C. EMERSON, M.D., DETROIT, MICHIGAN

THE tremendous shock which follows the escape of bile into the peritoneal cavity has been repeatedly observed. Bunting and Brown<sup>1</sup> investigated this phenomenon by either injecting bile intraperitoneally or by dividing the gall bladder of rabbits and allowing the bile to escape into the peritoneal cavity. Death generally occurred in twenty-four hours and was attributed to direct action upon the myocardium.

A general bile pigmentation of increasing intensity may be seen in early obstructive jaundice clinically and there may be no accompanying symptoms. The picture is entirely unlike that seen in bile peritonitis. However, in each syndrome bile is the underlying etiologic factor. It would therefore seem that in obstructive jaundice the bile in the blood stream may be detoxified or there may be a depression of the liver function and all the normal elements of bile may not be escaping into the circulation. Mann and his coworkers<sup>2</sup> having shown the extra-hepatic formation of bile pigment, the degree of jaundice would not necessarily be an index of the actual amount of whole bile present in the blood stream and the tissues.

King and Stewart<sup>3</sup> investigated the effect of the injection of pig's bile upon the circulation of dogs and concluded that the toxicity was due to the pigment. They further observed that the blood calcium rose 20 per cent during the experiments. They believed that the rise in blood calcium was a protective mechanism in that calcium combined with the pigment and rendered it nontoxic. It was further concluded that the delayed coagulation time in obstructive jaundice was due to the calcium being combined, and there was none available for the process of coagulation.

Lee and Vincent<sup>4</sup> demonstrated the importance of calcium administration by mouth and intravenously to shorten the coagulation time of the blood in obstructive jaundice. Walters has called attention to the fact that the high postoperative mortality rate in jaundiced patients was largely due to hemorrhage. He emphasized the importance of adequate preoperative preparation of such patients by the administration of calcium.

In view of the difference in the reaction of the organism to bile in obstructive jaundice and in bile peritonitis it seemed desirable to determine whether calcium had a true protective effect against toxicity. Were this demonstrated then the intravenous administration of a soluble calcium salt would be indicated as a preoperative measure in all cases of suspected bile peritonitis.

It would seem that if calcium were protective against the toxicity of bile

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this might readily be demonstrated by determining the lethal dose of a given sample of bile injected at a uniform rate of speed and repeat the experiment but increase the available calcium by the simultaneous injection of a soluble calcium salt to determine whether the lethal dose was altered

Meltzer and Salant<sup>1</sup> have shown that much of the confusion in the literature upon the relative toxicity of bile depended entirely upon the varied speed of injection used by different investigators. In all of our experiments we injected the bile at a uniform rate of speed 25 to 3 c.c. per minute

## METHOD

Ox bile was obtained from twenty gall bladders of freshly killed animals. The material was diluted with an equal volume of normal saline and injected within three hours after its collection. Dogs were used in all the experiments and an effort was made to obtain animals of approximately the same weight to test a single sample of bile. The dogs were anesthetized by the open drop method and later a cannula was placed in the trachea and connected with an ether bottle. The femoral vein was exposed and a Hartman two way needle inserted. This was connected to two burettes containing the injection material. The diluted bile was placed in one, and the calcium chloride solution or normal saline in the other depending upon the experiment. A 5 per cent solution of calcium chloride was used and the total amount to be given was injected simultaneously with the first 50 c.c. of bile. In the control animals an equal amount of normal saline was given. A cannula was placed in the carotid artery and connected with a mercury manometer for blood pressure tracings. A tube was placed in the etherization system and connected with a tambour

TABLE I

BILE SPECIMEN	WEIGHT OF CONTROL DOG POUNDS	LETHAL DOSE BILE PER POUND C C	WEIGHT OF DOG POUNDS	CALCIUM CHLORIDE PER POUND	LETHAL DOSE OF BILE PLUS CALCIUM CHLORIDE PER POUND C C
	pounds	c c	pounds	mg	c c
5	18 0	10 2	24 25	10 4	9 0
1	18 75	10 5	23 25	10 9	7 6
4	21 5	6 8	23 0	10 9	5 8
2	19 75	9 7	21 25	11 9	8 7
2	19 75	9 7	16 25	16 0	8 6
2	19 75	9 7	15 25	16 6	8 6
3	11 75	6 9	14 5	18 0	7 2
1	18 75	10 5	13 0	19 0	9 2
3	11 75	6 9	8 5	29 4	8 1
7	15 75	8 8	16 75	30 0	6 3
6	12 5	8 4	16 5	30 3	8 3
6	12 5	8 4	7 5	33 3	8 0
5	18 0	10 2	13 75	36 3	6 3
7	15 75	8 8	13 0	38 4	8 5
5	18 0	10 2	12 5	40 0	7 2
7	15 75	8 8	11 25	44 4	6 0
5	18 0	10 2	10 0	50 0	6 5
7	15 75	8 8	10 0	50 0	5 4
6	12 5	8 4	9 75	51 3	8 2
Average		8 7			7 5



and writing lever for a tracing of the respiratory movements. Blood pressure and respiration tracings were made after the injection of every 25 cc of diluted bile.

### RESULTS

The lethal dose of seven samples of bile was determined in control animals and the effect of the simultaneous introduction of calcium chloride in varying dosage from 10 to 52 mg per pound of body weight was observed. It was not deemed advisable to inject greater quantities of calcium chloride because the usual therapeutic dose in man is 4 mg per pound and according to Walters and Bowler<sup>8</sup> the lethal dose for a dog is 1163 mg per pound.

Fig 1 shows the characteristic gradual fall of blood pressure which occurs when bile is injected into a systemic vein. There is a striking arrhythmia and at times an apparent cessation of cardiac activity.

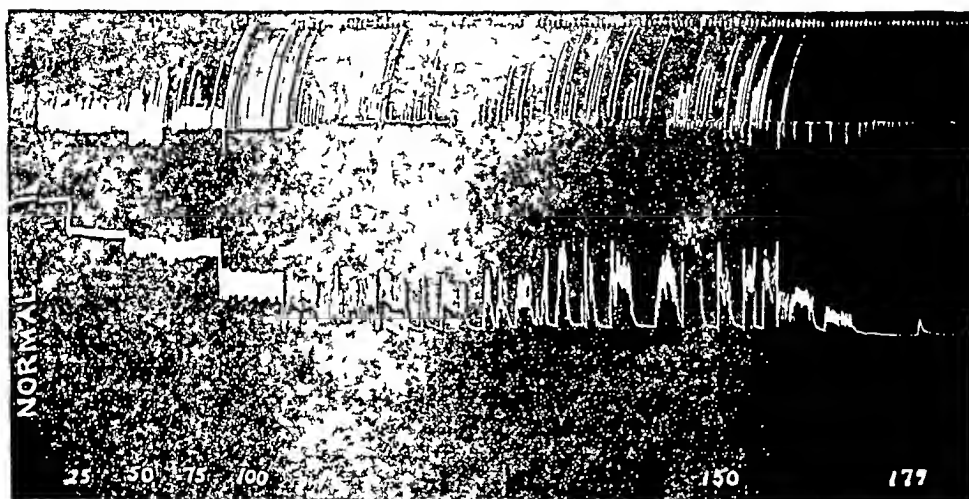


Fig 1—Effect on the blood pressure and respiration when bile is injected into the femoral vein.

Fig 2 shows essentially the same picture. The dog weighed 13 pounds and was given 19 mg of calcium chloride per pound. There is a similar fall of blood pressure and arrhythmia to that previously noted.

The results are summarized in Table I. The lethal dose in the control animals varied from 6.8 to 10.5 cc per pound while the average lethal dose for the group was 8.75 cc per pound.

The lethal dose of bile in 19 dogs which received calcium chloride varied from 5.4 to 9.2 cc per pound. The average lethal dose for this group was 7.5 cc per pound.

### DISCUSSION

The experiments demonstrate that the simultaneous intravenous injection of calcium chloride in varying dosage with fresh ox bile definitely does not decrease the toxicity of the bile. Although in both series of experiments the injections were made at a uniform rate, the lethal dose for those animals

which were given calcium chloride was somewhat less than in the controls. As the dosage of calcium was increased the difference was more striking. This is of interest because of the observations of Walters and Bowler<sup>5</sup> that the lethal dose of calcium chloride in a jaundiced dog was much greater than in a normal animal.

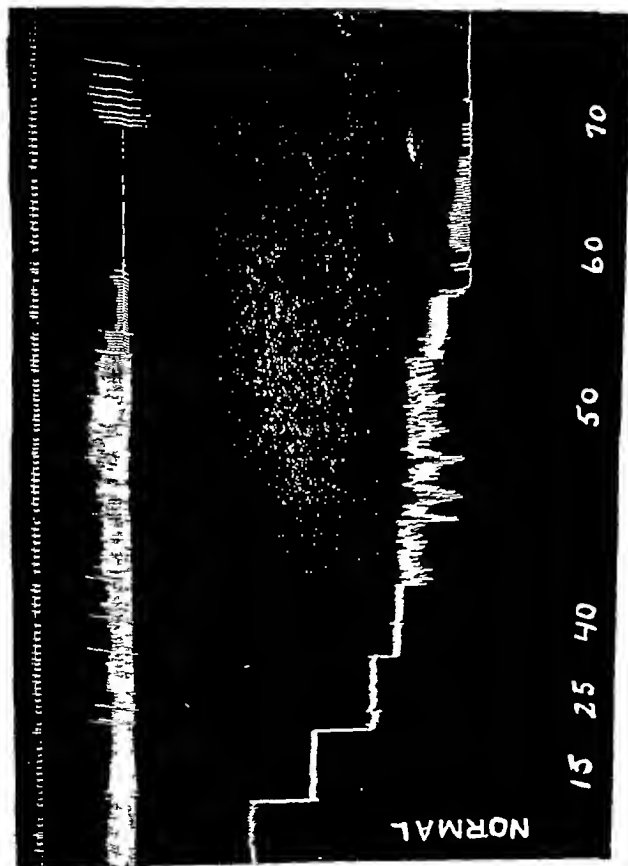


Fig. —Effect on the blood pressure and respiration when bile and calcium chloride 19 milligrams per pound were injected into the femoral vein

It would seem that the injection of bile resulted in the mobilization of calcium which enters the blood stream. The delayed coagulation time in obstructive jaundice may be due to the calcium being in a combined form and not being available for the process of blood clotting. However, the experi-

ments presented do not suggest that the calcium is combined with the toxic element or elements of the bile but rather to some other constituent, possibly the protein, and therefore is not protective

### CONCLUSIONS

1 Calcium chloride shows no protective effect against the toxicity of bile in the normal animal in doses varying from 10 to 52 mg per pound of body weight

2 The beneficial effect of calcium chloride in obstructive jaundice as pre-operative preparation would seem to be limited to its effect upon the coagulation time of the blood rather than a true detoxifying action

3 There is nothing to suggest that calcium chloride would be beneficial in fulminating bile peritonitis

This work was suggested and done under the direction of Dr Edward C Davidson

### REFERENCES

- <sup>1</sup>Bunting, C H, and Brown, W H The Pathology of Intraperitoneal Bile Injections in the Rabbit, *Jour Exper Med*, 1911, **xx**, 445
- <sup>2</sup>Munn, T C, Sheard, C, Bollman, J L, and Baldes, E J The Site of the Formation of Bilirubin, *Am Jour Physiol*, 1925, **xxv**, 497
- <sup>3</sup>King, J H, and Stewart, H A The Effect of the Injection of Bile on the Circulation, *Jour Exper Med*, 1909, **xi**, 673
- <sup>4</sup>Lee, R J, and Vincent, B The Relation of Calcium to the Delayed Coagulation of Blood in Obstructive Jaundice *Arch Int Med*, 1925, **xxv**, 59
- <sup>5</sup>Walters, W Pre operative Preparation of Patients With Obstructive Jaundice, *Surg, Gynec and Obst*, 1921, **xxxi**, 657
- <sup>6</sup>Meltzer, S J, and Salant, W Studies on the Toxicity of Bile, *Jour Exper Med*, 1905, **xv**, 280
- <sup>7</sup>Hartman, F W Transfusion Reactions and Citration Within the Needle, *Jour Am Med Assn*, 1922, **lxxviii**, 15
- <sup>8</sup>Walters, W, and Bowler, J P Pre operative Preparation of Patients With Obstructive Jaundice Experimental Study of Toxicity of Intravenous Calcium Chloride Used in Preparation of Patients, *Surg, Gynec and Obst*, 1904, **xxix**, 200

## THE ACTION OF COLD UPON THE T WAVE OF THE ELECTROCARDIOGRAM\*

HAROLD L. OTTO, M D, NEW YORK

**I**N 1909 Eppinger and Rothberger<sup>1</sup> found that when the basal and right portion of the heart of the dog is chilled by the ethyl chloride spray the chilling exerts a positive influence upon the T-wave of the electrocardiogram. When it is applied to the apical and left portions of the heart, the opposite effect appears, and a negative influence upon the T-wave occurs. These observations upon the effect of cold upon the T wave were confirmed by the work of Wilson and Herrmann,<sup>2</sup> and Smith,<sup>3</sup> the former also pointing out

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<sup>3</sup>Smith did not use ethyl chloride in his experiments because of Lewis' objection that it throws the muscle involved out of function.

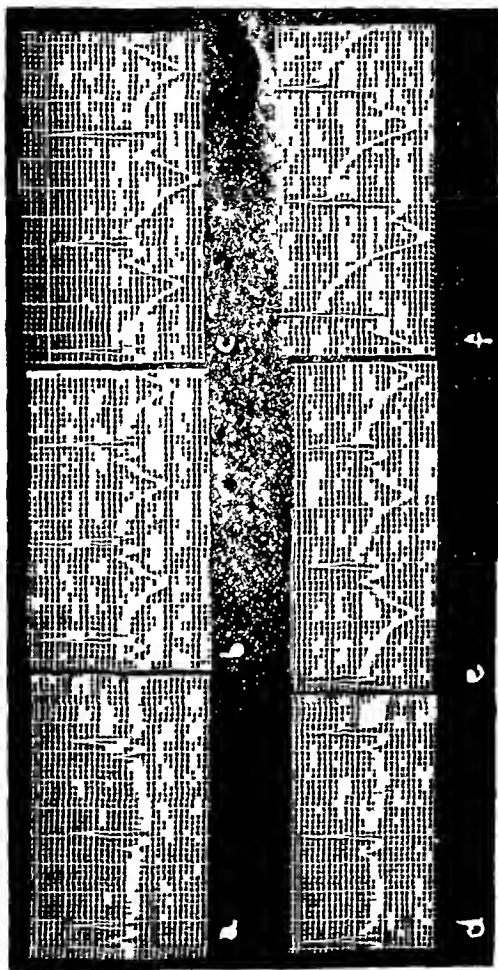


Fig. 1.—Electrocardiogram of the heart of a cat using axial (right arm—left leg) leading. Time in fiftieth seconds. *a* The normal electrocardiogram. *b* and *c* During the application of the ethyl chlorid spray to the outer wall of the right ventricle. Great negativity of the T wave ensues. *d* After the curve has returned to the normal. *e* and *f* During the application of the spray to the outer wall of the left ventricle. Note the negative action upon the T wave which precedes the final effect.

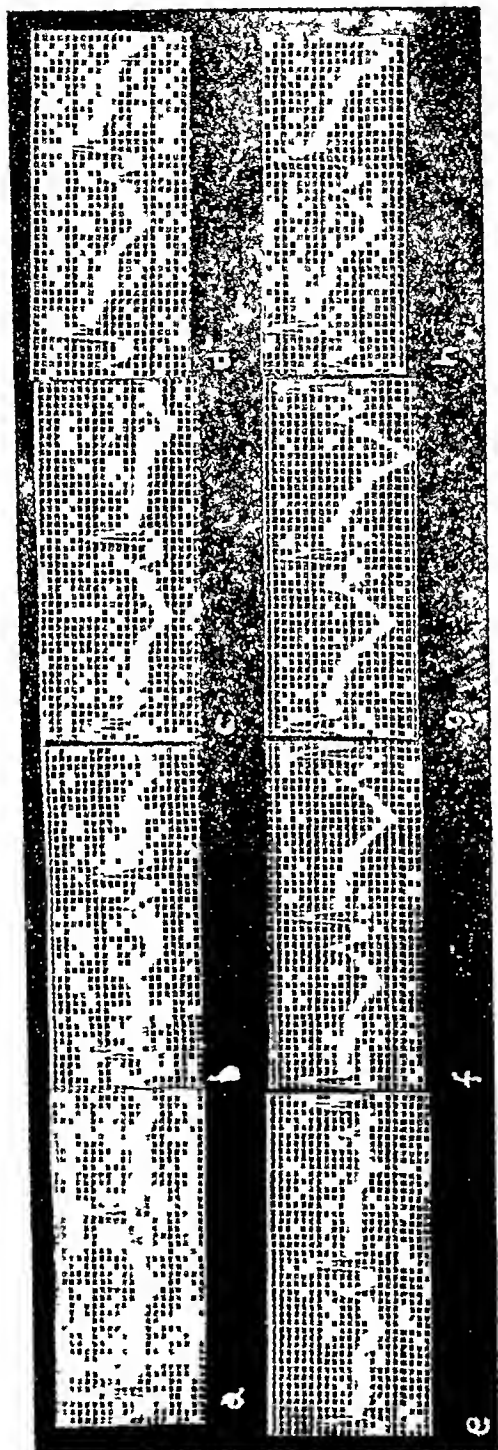


Fig 2.—The same animal as Fig 1. *a* The normal electrocardiogram. Slight changes have occurred in its form undoubtedly due to the action of the ethyl chloride spray. *b*, *c* and *d* During the application of ice to the outer wall of the right ventricle. Note that the effect is the reverse of the effect obtained with ethyl chloride. *e* Ten minutes after the ice has been removed from the heart. *f*, *g* and *h* During the application of the ice to the outer wall of the left ventricle. Observe how the negative influence upon the T-wave in *g* passes into the reverse effect in *h* as the application is prolonged. Compare this with Fig 1 *c*.

that since the effect of cold is to prolong the processes involved in muscle contraction, the T wave changes induced agree with the assumption that it represents the decline of the active process in the muscle contraction. That this is the same for the human heart has been pointed out by Wilson and Finch.<sup>4</sup> These authors obtained cooling of the posteroinferior aspect of the apex of the left ventricle by having the subjects drink iced water.

When the ethyl chloride method is repeated upon the heart of the cat, these effects are exactly reversed, and the freezing of the basal right portion of the heart causes marked negativity of the T wave, and freezing the apical left portion of the heart causes marked positivity of the T wave (Fig 1). If, however, the chilling of the heart is made less intense than that produced by the ethyl chloride spray, e.g. by the application of cold water or ice, the effects occur as these authors describe them and tend to reverse themselves as the action of the ice is prolonged (Fig 2). Since the external ventricular walls in these areas of the cat's heart are sufficiently thin to be frozen by the ethyl chloride, there can be little doubt that the procedure throws the portion of the wall involved entirely out of function.

Although the effects in all the experiments\* were not always pure the contrast of the results under the two sets of conditions, prolongation of and elimination from function, indicates that in the axial lead, actual right basal effects are associated with a positive influence upon the T wave since prolongation of its activity, or lessening of left apical ventricular effects elevate the T wave, and actual left apical effects are associated with a negative influence upon the T wave, since prolongation of its activity or throwing muscle of the right ventricle out of function causes negativity of the T wave. Furthermore they suggest that when the summation of the events of the cardiac contraction are such as to favor the right ventricle by a prolongation of its activity (temporal preponderance) or an algebraic increase in the muscle activity over that of the left side of the heart (volume preponderance) the T wave tends to positivity, and in the case of the left side of the heart, the reverse action or negativity of the T wave is the result. There is, however, no sensitivity in this balance of the muscle activity of the right and left ventricles inasmuch as a comparison of the effects of ethyl chloride in the heart of the dog and cat points out that the area involved must be large in its relation to the size of the heart before the appearance of an easily recognizable effect from a volume preponderance in the axial lead since in the dog the spraying indubitably throws muscle out of function without the electrocardiogram showing the effect expected.

#### REFERENCES

- 1 Eppinger H. and Rothberger, J. *Wien klin Wchnschr* 22 1091, 1909
- 2 Wilson, F. N., and Herrmann G. R. *Heart* 2 229, 1921
- 3 Smith, F. M. *Heart* 10 391, 1923
- 4 Wilson F. N., and Finch, R. *Heart* 10 215, 1923

\* There were 10 trials in 5 cats under ether anesthesia the order of investigation being ethyl chloride to right then left ventricles then ice or ice water flowing through a fine tube to the right then left ventricles with a sufficient interval between each step for recovery. The heart was always carefully protected with absorbent cotton so that only the area to be tested was exposed.

## POSITIVE BLOOD CULTURES IN NONSPECIFIC ULCERATIVE COLITIS\*

By BURRILL B. CROHN, M.D., AND GREGORY SHWARTZMAN, M.D., NEW YORK

THE subject of nonspecific ulcerative colitis has given rise in recent years to considerable discussion. It would seem that, as a clinical entity, this type of colitis is just assuming its real significance. The clinical symptomatology is not yet complete. New phenomena are still being described. Conceptions regarding the course and prognosis are in a state of continuous change. The treatment surely is in perpetual flux, varying between absolute conservative medical management to extreme radical surgical methods. Probably the least determined point is the etiology and in this connection particularly the rôle that streptococci singly or in symbiosis play as a possible causative factor. It is particularly as bearing on the point of probable bacterial etiology of this disease that we offer this contribution dealing with cases of nonspecific ulcerative colitis in which positive blood cultures have been found. The recovery of positive blood cultures in this disease constitutes to the best of our knowledge an original observation.

CASE 1—A girl (E. S.) nineteen years of age, who two years previously had an attack of diarrhea with bloody stools. She had been severely ill for seven weeks under hospital treatment and was discharged improved. She remained well until five months before admission when she had a recurrence of her trouble, many daily movements containing blood, loss of appetite and of weight. Under intestinal irrigations and dieting, a certain amount of improvement took place. She was admitted, however, to Mount Sinai Hospital with the statement of having five to six bloody movements a day and of experiencing sharp, radiating pain with each bowel movement. She was pale, thin, undernourished, the abdomen was relaxed. There was marked tenderness over the course of the colon. The blood count showed a marked secondary anemia, hemoglobin 42 per cent, color index of 0.48 and a normal differential count. The stools were bloody and contained no parasites and no amebae. She had, however, a small perforation of the nasal septum and radiographically showed a mild clouding of the left ethmoid sinus.

By sigmoidoscopic examination, the mucous membrane was seen to be granular, hypertrophic, friable, bleeding easily and showing innumerable small pin-point hemorrhages, but no definite ulcerations. The temperature on admission was 104° F., a febrile course at this level continued for several days but, in the course of two or three weeks, showed a gradual subsidence to almost normal. Two weeks after admission she suffered pain in the left lower extremity, on physical examination a bluish pallor of the left leg and toes was seen, tenderness was present along the left femoral vein. A tentative diagnosis of phlebitis and thrombosis of the left femoral vein was made. During the course of the next three or four days, the swelling of the left leg increased and then subsided without further physical signs.

Among the interesting clinical symptoms observed in this patient, there was a burning and painful sensation in the mouth and on the tongue. On examination one noted a moderately severe aphthous stomatitis, consisting of a small vesicular eruption scattered over the buccal mucosa and gingival surfaces of the tongue.

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The course of the case was favorable, a constant and steady improvement taking place. One month after admission sigmoidoscopy still showed an advanced stage of severe colitis with innumerable ulcerations pinhead to pencil tip in size. Acriflavin irrigation treatment was begun at this point. Some further swelling of the left leg took place without elevation of temperature. A definite diagnosis of femoral thrombosis could not be made though the symptoms were suggestive. The eruption on the oral mucosa continued for some time, the patient showing persistent evidence of small ulcers on the inferior surface and lateral margins of the tongue. The secondary anemia persisted, hemoglobin showing 51 per cent and red blood count 3,400,000 cells. In the course of the next few weeks the patient made a steady improvement. Small doses of vaccine obtained from Dr. J. A. Bergen of the Mayo Clinic were given, accompanying the acriflavin irrigation treatment. The patient was finally discharged two months after admission very definitely improved.

**Bacteriologic Data**—Several bacteriologic examinations of the stools were made and reported as follows:

July 10, bacillus coli and *Streptococcus hemolyticus*

July 17, enterococcus and bacillus coli

July 18, bacillus coli communis and *Streptococcus hemolyticus*. Pus examination directly from the sigmoid mucosa showed enterococcus.

An aerobic blood culture taken on admission was negative. A routine aerobic blood culture taken eight days later during the height of the febrile course showed an *hemolytic streptococcus* growing in the fourth and fifth flasks.

**CASE 2**—In brief this is a history of a sixteen-year-old school girl (P. G.) who was admitted to the Mount Sinai Hospital complaining of a diarrhea of five months' duration. She had had from three to nine movements a day, the stools being loose, watery, and containing blood and mucus. She had lost ten pounds in weight, complained of weakness, increasing pallor, and dry cough. Physical examination showed a small area of bronchopneumonia in the left interscapular region and a few rales at both pulmonary bases. There was a secondary anemia, hemoglobin being 50 per cent, red count 2,300,000, and white count 15,000, the only unusual characteristic being an eosinophilia of 6 per cent. The temperature on admission ranged between 104° and 105° F. A systolic murmur was present at the apex of the heart, the murmur presumably being of hemie origin.

Sigmoidoscopic examination showed a markedly reddened mucosa with hemorrhagic infiltrations, but no definite ulcers. The membrane was friable and profuse bleeding followed the examination.

Within two or three days after admission it was noted that herpes was present on the lips. This herpes persisted for a few days and then gradually disappeared. The retinal field showed a few scattered petechial hemorrhages on several examinations.

The patient showed continuous improvement under treatment; the temperature subsided rapidly, but showed unaccountable irregular rises during the two months that the patient was in the hospital. Under acriflavin retention enemas, and forced general diet the patient at the end of eight weeks was practically well and was discharged from the hospital.

**Bacteriologic Data**—An aerobic culture was made upon this patient the day of admission at the time of the highest temperature (104° F.). A *Streptococcus hemolyticus* of the mucoid type was found in the fluid medium of the flasks. Subsequently, an anaerobic blood culture was reported negative. Agglutination tests of serum against strains of dysentery organisms were persistently negative.

In this case one positive culture of blood contained an *hemolytic streptococcus*. A mild bronchopneumonia was present at the time; there was, therefore, some doubt as to whether the colitis or the pneumonia was accountable for the blood stream invasion. Our experience has been that negative blood cultures are routinely obtained in ulcerative colitis as well as in mild cases of bronchopneumonia. The febrile reaction was quite evidently due to the severity of the colitis because it continued irregularly for several weeks after the subsidence of the mild pneumonic signs and accompanied the fluctuations and severity of the colitis symptoms.



CASE 3—(J K) A man of twenty five years under observation on the wards of Mount Sinai Hospital for almost nine months. He had been suffering with symptoms of a severe colitis at irregular intervals during the preceding four years, during which time he had lost seventy pounds in weight. In May, 1927, an ileostomy had been performed with the hope of causing a reduction in temperature and improvement in the course of the disease. However, in October, five months after the operation, the patient was transferred to the medical wards for our care, the ileostomy having succeeded only in some slight general improvement. At this time sigmoidoscopy showed a severe generalized ulceration, with marked proliferative hyperplasia and polypoid regeneration. While under our observation he was treated with a vaccine furnished by Dr J A Barger of the Mayo Clinic. He received also gentian violet by mouth, irrigations with acriflavin and at other times with flumerin. Some weeks later we administered, intravenously, large doses of polyvalent antidyentery serum, which resulted in a slight immediate benefit, but no marked change in the course of the disease. On the basis that any benefit derived from vaccine or serum was in the nature of a nonspecific reaction, we gave the patient a series of typhoid vaccine injections, employing sufficient amounts to cause severe reactions.

The course of the disease was, however, slowly but progressively downward. Irregular temperature continued, symptoms of arthritis of the large joints supervened. At one time there was severe pain in the left perineum and in the tuberosity of the ischium, so that we suspected a perianitis. Radiographic studies were negative. Eventually, in desperation, a colectomy was suggested, the sigmoidoscopic picture giving one an impression that the damage to the mucosa was so extensive and the clinical course so hopeless that only a radical removal of the diseased tissues could offer any permanent hope. A colectomy was performed which resulted fatally five days after operation, due to the marasmic condition of the patient and a postoperative infection of the abdominal wall.

On various occasions cultures obtained from the wall of the intestines through the sigmoidoscope showed bacillus coli, bacillus pyocyaneus, and on two occasions an enterococcus. One blood culture taken in November was negative, but the two blood cultures taken later at the peak of a rise in temperature showed enterococci in the fluid culture media. Another blood culture taken four months after these findings was negative.

#### TECHNIC OF BLOOD CULTURE EXAMINATIONS

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The positive findings in fluid media alone were obtained in conditions which could not be termed septicemias. They were in the nature of transient bacteremias. Septic foci communicating freely with the general circulation could not be detected clinically nor were such conditions found at post mortem examination. In many of the cases there were no clinical signs which would point to the presence of bacteria in the general circulation. Frequently, a rise of temperature for a day or two with subsequent speedy recovery was the only symptom. Positive blood cultures in fluid media were also obtained on several occasions in acute rheumatic fever, debilitating conditions such as malignant tumors, tuberculosis, acute anterior poliomyelitis and other similar conditions. The organisms found in this group of cases were mainly streptococci, mostly nonhemolytic and sometimes green producing streptococci.

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#### CONCLUSIONS

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2 Possible interpretations of such findings are developed but it is recognized that considerable experimental and clinical work is necessary before definite conclusions can be drawn.

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Duke<sup>1, 2</sup> has reviewed the advances in our knowledge of allergy in the last two years. However, because of the breadth of the scope of his papers, he has been unable to put much stress on the subject of hay fever. Although nothing radically new has occurred in this field in the last two years, it is gratifying to note nevertheless that considerable progress has accumulated in our knowledge of the etiology and treatment of this disease.

#### ETIOLOGY AND PATHOLOGY

Although many reports appear in the literature concerning the causes of hay fever as a phenomenon of sensitization to pollen, most of these are repetitions of facts already recorded in the past.<sup>3</sup> The all important question of the basic mechanism underlying the predisposition to sensitization has not yet been answered. In the meanwhile, however, there have appeared several articles which throw considerable light on heredity and other factors in the etiology.

Clarke et al.,<sup>4</sup> in a study of heredity in allergy, show that not only is the allergic tendency inherited but also the organs or tissues involved are more or less predetermined by heredity. They present statistics showing that pure asthmatic subjects have more pure asthmatic antecedents than pure hay fever antecedents, and that pure hay fever subjects have more pure hay fever than asthmatic antecedents.

Balver<sup>5</sup> in a study of the factors governing the acquisition of hypersensitivity, concludes that inheritance is the principal factor determining whether or not an individual will develop clinical hay fever or asthma, and chiefly governs the time of life when symptoms appear. He also maintains that heredity influences the kind of atopy to which the individual will become sensitive. The earlier in life sensitiveness occurs, the greater the likelihood of becoming sensitive to more than one group of atopogens. Degree of exposure to a given substance is also a deciding factor whether sensitiveness will develop to that substance. Balver has demonstrated also that a child may be born specifically sensitive to a food protein, sensitization occurring probably by placental transmission of atopogens.

In a series of 36 individuals who had an hereditary background of hay fever, Baldwin<sup>6</sup> has attempted to determine the relation of the onset of the cutaneous sensitivity to the clinical symptoms. Of this number five developed positive skin tests simultaneously with the development of hay fever, in a sixth case of hay fever the skin tests did not become positive until the second season.

Bristow<sup>7</sup> says that "It (allergy) seems to be a disease of civilization, hay fever among negroes is rare." This is not at all in accord with the experiences of the reviewer who sees many cases of hay fever and asthma among negroes in the Clinic. This is substantiated further by recent reports of considerable

CASE 3—(J K) A man of twenty five years under observation on the wards of Mount Sinai Hospital for almost nine months. He had been suffering with symptoms of a severe colitis at irregular intervals during the preceding four years, during which time he had lost seventy pounds in weight. In May, 1927, an ileostomy had been performed with the hope of causing a reduction in temperature and improvement in the course of the disease. However, in October, five months after the operation, the patient was transferred to the medical wards for our care, the ileostomy having succeeded only in some slight general improvement. At this time sigmoidoscopy showed a severe generalized ulceration, with marked proliferative hyperplasia and polypoid regeneration. While under our observation he was treated with a vaccine furnished by Dr. J. A. Bargen of the Mayo Clinic. He received also gentian violet by mouth, irrigations with acriflavine and at other times with flumerin. Some weeks later we administered, intravenously, large doses of polyvalent antidysentery serum, which resulted in a slight immediate benefit, but no marked change in the course of the disease. On the basis that any benefit derived from vaccine or serum was in the nature of a nonspecific reaction, we gave the patient a series of typhoid vaccine injections, employing sufficient amounts to cause severe reactions.

The course of the disease was, however, slowly but progressively downward. Irregular temperature continued, symptoms of arthritis of the large joints supervened. At one time there was severe pain in the left perineum and in the tuberosity of the ischium, so that we suspected a perianostitis. Radiographic studies were negative. Eventually, in desperation, a colectomy was suggested, the sigmoidoscopic picture giving one an impression that the damage to the mucosa was so extensive and the clinical course so hopeless that only a radical removal of the diseased tissues could offer any permanent hope. A colectomy was performed which resulted fatally five days after operation, due to the marasmic condition of the patient and a postoperative infection of the abdominal wall.

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hay fever in South Africa, and asthma in India. It would seem that in those countries in which hay fever is rare, it is more a matter of botanic than racial considerations.

Vaughan<sup>8</sup> makes the observation that Weil maintains that the difference in quantitative distribution of smooth muscle among different experimental animals accounts for their different shock organs: rabbit, pulmonary arterioles, guinea pig bronchi, dog, liver, etc. This is offered as a possible explanation by Vaughan as to why some allergies develop hay fever, others asthma, and still others cutaneous manifestations. He also considers the following etiologic factors of importance, especially from the standpoint of treatment, the conception that pollinosis is a constitutional disease, that specific local sensitization may play some rôle, that factors deciding nasal localization may be nonspecific such as infection and mechanical obstruction, and that coexistent sensitization to food or other inhalants may aggravate or localize the hay fever.

Balveat<sup>9</sup> emphasizes the importance of this root in allergy. In 373 cases of hay fever he has found it to be a factor in 72 per cent. He says that the majority of these virus-sensitive individuals must be desensitized.

Duke<sup>10</sup> has found that in hay fever cases 10 per cent had nasal polyps and 6 per cent sinus infection. He considers this secondary to allergy rather than primary, and warns against nasal operations in such cases. In a study of 91 patients who had a nasal complaint in addition to their presenting symptoms, Everman<sup>11</sup> found that in the hypersensitive cases 72 per cent showed eosinophiles in the nasal secretions, while in the nonallergic cases only 9 per cent showed occasional eosinophiles.

Grafton Tyler Brown<sup>12</sup> made an analysis of the eosinophile counts in asthma, hay fever, and hyperesthetic rhinitis. His conclusions are that in asthma the average eosinophilia is as high in allergic as in the nonallergic cases and that in hay fever and hyperesthetic rhinitis the blood eosinophilia is equally high and presents no difference in the sensitive and nonsensitive cases. As a result of a study of eosinophilia at various stages of hay fever Sternberg<sup>13</sup> suggests that the increase of eosinophiles in hay fever seems to be a shock reaction. He calls attention to the fact that no increase was observed in patients free from symptoms before and during their treatment.

In a series of 85 cases studied, comprising 53 cases of asthma, 20 of hay fever and 12 nonseasonal allergic rhinitis Sterling<sup>14</sup> finds a hypocalcemia in only 23 while phosphorus deficiency (0 to 30 mg per 100 cc) was present in 68.

As in the past one still finds in the literature allusion to fanciful, unsubstantiated hypotheses attempting to explain the pathology and treatment of hay fever. Thus, Leach<sup>15</sup> claims that all sensitization, hay fever, asthma, etc., is a result of nasal pathology. He maintains that the nose acts as a filter to prevent the entrance of toxic substances into the blood, that when nasal pathology is present filtration is not adequate, hence asthma or hay fever. Bird<sup>16</sup> states that hay fever is due to pathologic currents (electrical?) which may enter the nose via the nasal ganglia or nasal nerves. This author tries to

explain almost every disease by the conception of pathologic currents in which the brain is the distributing center and abnormal conditions result from 'shorts' of excessive currents

#### CLINICAL MANIFESTATIONS AND DIAGNOSIS

According to Balveat<sup>12</sup> the general health of the hay fever patient is better than the average and he has a greater resistance to infectious disease. This is true in spite of the fact that in the autumnal hay fever cases 28 per cent had asthma during the season and 60 per cent tend to develop asthma eventually.

Kahn<sup>17</sup> states that in chronic hay fever or asthma in the South there may be in addition to the allergic symptoms a chronic toxemia due to pollen. This occurs mainly in children and consists principally of retarded growth backward mentality, undernutrition, sallow skin and enuresis.

Pollen hay fever and asthma in children does not seem to have the adult picture, according to Kahn<sup>18, 19</sup> it is usually mild and is frequently mistaken for symptoms of adenotonsillar hypertrophy. He says that a definite bronchitis, accompanying the rhinitis, precedes the initial asthma. At that stage the allergic nature of the manifestations may be recognized by coexisting vasomotor rhinitis, pollen precautions and relief with epinephrin. High fever and leucocytosis often accompany the asthmatic attacks and may lead to a diagnosis of pneumonia. To this the reviewer would like to add that similar errors arise in asthma in children due to things other than pollen. Many of the acute respiratory conditions in children diagnosed as pneumonia, tonsillitis, or infectious bronchitis are either asthma or allergic bronchitis. Other essential differences in pollinosis of children and adults as noted by Kahn are the mildness of the nasal symptoms in children, the definite toxemia frequently occurring and the less marked diagnostic skin tests.

Lamson,<sup>6</sup> in a series of intracutaneous tests with pollens in over 100 hay fever cases shows a distinct specificity as to species and genus. Many of the papers, such as that of Hirtwitz,<sup>1</sup> corroborate further the diagnostic value of skin tests. Kahn again reiterated his previous statements in regard to the unreliability of skin tests. He claims that negative skin tests both cutaneous and intracutaneous are so frequent in the presence of pollinosis as to make such tests undependable. He advocates the use of subcutaneous tests daily increasing injections subcutaneously until a reaction is obtained. He admits, however, that he may have to push the injections to as high a concentration as 1:20 before reactions occur. He states<sup>2</sup> that negative skin tests (with as high as 2 per cent pollen intradermally) are frequent in Texas in pollen asthmatics in infants and young children especially. He adds, however, that the longer the duration of asthma the less likelihood is there of negative skin tests. It may be said that Kahn's experiences are not in accord with those of the reviewer nor with those of the majority of allergists. However, peculiar climatic conditions in the South may possibly account for this difference.

In a report of further observations on the nonspecific diagnosis of allergy I<sup>24</sup> have presented a series of cases of asthma, hay fever and perennial rhinitis in whom a diagnosis of allergy could be made by the intradermal test with

human dandel extract The results in hay fever, however, were not as good as in asthma in 38 cases 36 reacted with the dandel extract Although this reaction is of no great practical value in hay fever, it is of great value in perennial rhinitis or asthma

#### EXPERIMENTS WITH ATOGENS AND ATOPIC REAGENS

Lamson Piness, and Miller<sup>20, 26, 27</sup> in a series of experiments with spring and fall hay fever cases, showed that there is a definite diminution of skin reactivity after pollen treatment They say that skin desensitization to fall pollens results in a majority of cases after the usual course of treatment This persists at least for four weeks and in some cases for months or years In a study of a number of allergic patients in whom negative reactions were obtained to certain pollens the injection of such pollens did not sensitize the skin They also failed to engender skin reaction to certain pollens by injecting them into a person definitely hypersensitive to other pollens From this they rightly suggest that natural exposure, inhalation of pollens, or injection of the same pollens, are not the only factors necessary to induce skin hypersensitiveness in an allergic person

On a large number of pollen-sensitive patients Huber<sup>12</sup> made a study of the skin reactions to dry large and small ragweed pollen grown in different sections of the United States The samples of the same species of pollen varied considerably in color due probably to the method used in collecting and preserving them With the same species of pollen some patients consistently gave greater reactions to one or more of the samples than to the others, while other patients gave greater reactions to different samples Huber interprets this variation in reaction to mean that the chemical composition of the samples varied and that all patients were not sensitive to the same constituent

The disputed question of the identity of the atogens of the short and giant ragweed has been studied further by Aaron Brown<sup>28</sup> The latter maintains that these atogens are entirely identical, and he supports his claims by the following observations In a series of 31 late hay fever subjects tested with extracts of both giant and short ragweed they were found to be equally sensitive to both One group was given a series of prophylactic treatments with one extract and the other with the other with results as good as in combined treatment After a maximal tolerance had been established for one extract an equivalent or larger quantity of the other extract was injected without being followed in any case by a constitutional reaction

In experiments on rabbits the Scandinavian authors, Thomsen and Baage<sup>29, 30</sup> have found that pollen injections caused a high antibody titer This was quite specific for the species but showed quantitative relationship to members of the genus of pollens In such highly immunized animals they found no evidence of sensitization as could be determined by intracutaneous or subcutaneous injections or conjunctival application On the other hand, Loeb<sup>31</sup> was able to sensitize guinea pigs with watery extracts or alcohol precipitates of pollen (*Pinus silvestris*) and goose feathers

Grove<sup>32</sup> attempted to produce local hypersensitiveness in lower monkeys and chimpanzees by the method of passive transfer originally described by

Prausnitz and Kuster and found to be highly successful in experiments on man. In this work the pooled serum containing the atopic reagins for ragweed, horse serum, L<sub>1</sub> Page's gluc and cottonseed were used. Her conclusions are that the results are practically negative as regards the production of local or constitutional hypersensitiveness.

Black<sup>33</sup> cites several reasons for throwing doubt upon the relation of the atopic reagin to the true atopen in pollen. He says that negative skin reactions may occur in clinically sensitive cases and positive reactions may occur in absence of clinical sensitiveness. In the reviewer's experience this certainly occurs very rarely in this part of the country (Chicago district). Again, he says skin reactions may continue unchanged in individuals successfully treated, and they may persist long after spontaneous cure of the patient. The size and intensity of the skin reaction have no relation to the severity of the clinical condition. Finally he claims that in mixtures of reagin and atopen the reagin was neutralized while the atopen remained unchanged. As a whole his arguments are well founded and should stimulate further experiments in this field.

#### REGIONAL BOTANIC REPORTS

In the last two years there has been considerable progress in the accumulation of botanic surveys of hay fever plants in various parts of the United States and in some foreign countries. This demonstrates, more than any other fact perhaps, the general appreciation of the importance of the hay fever problem and its relation to the local flora.

G. T. Brown<sup>34</sup> reports on the late spring or summer hay fever in the District of Columbia and vicinity. He incriminates the following: sweet vernal grass, June grass, orchard grass, timothy, red top grass and English plantain. Bernton<sup>5</sup> records 3 cases of hay fever due to the pollen of the paper mulberry tree (*Papirus papyrifera* Knutze) in Washington, D. C. He says that this tree, although a native of Eastern Asia, grows in the eastern half of the United States from New York to Florida.

In the Colorado Springs district of Colorado Conway<sup>6</sup> reports the most important hay fever plants to be Russian thistle, lamb's quarter, sage ragweed and cottonwood tree. In addition to his complete and classic botanic survey reported on in 1926 Waring<sup>37</sup> reports seven cases in which the cotton of the cottonwood tree was probably a cause of "hay fever." Tests with pollens of the usual plants pollinating at that time of the year were negative while the intradermal injection of the cotton extract was positive. All of these patients were also sensitive to the pollen of the cottonwood.

In Oregon Chamberlain<sup>38</sup> considers 95 per cent of the hay fever to be of the midsummer type and caused by the grasses. The other plants of importance are Russian thistle and the artemesiae. From pollen counts and a botanic survey of the east shore of San Francisco Bay Rowe<sup>39</sup> concludes that in that region the grasses pollinate all year round, but are most profuse from March to August. The trees pollinate from January to July. *Ambrosia*, *artemesiae*, and *chenopodiae* are causes of hay fever, but are not very profuse. Of the *ambrosiae*, the Western ragweed (*Ambrosia psilotachya*) is the one found in that region.

Although it is now accepted opinion among the vast majority of allergists that brightly colored plants and flowers are not a cause of hay fever, Stewart,<sup>40</sup> writing from Iowa, seems to consider also the rose, dandelion, goldenrod, goldenglow, daisy, aster, dahlia and others as contributing factors. In western Montana, Foss<sup>41</sup> reports three hay fever seasons: spring (March to April) due to the trees, early summer (May and June) due to grasses, and late summer (July to frost) due to Russian thistle, sagebrushes and poverty weeds. The last group, according to the author, accounts for 60 per cent of all hay fever cases in that region. In Nevada a somewhat similar situation is reported by Albert<sup>42</sup> on the basis of skin tests made on 31 hay fever cases. He says that the Russian thistle, pollinating from July to October, is the most important hay fever plant, other chenopods, shad scale, annual salt bush, and lamb's quarter are also important. As a group he finds the grasses more important than the chenopods, and the amaranths of least importance.

Balyeat<sup>43</sup> surveys the botanical situation in Oklahoma and concludes that the important hay fever plants are Bermuda grass (May 15 to frost), amaranths (pigweeds), Western water hemp, and the ragweeds. In the middle southern states Spitz<sup>44</sup> reports the three seasons as they are known in the more northern states with the important additions, perhaps, of Bermuda and Johnson grass in the former.

A survey of the Salt River Valley in Arizona by Phillips<sup>4</sup> shows the false ragweeds, *franseria acanthocarpa*, *f tenuifolia*, and *f deltoidea*, to be of great importance. The Western ragweed (*ambrosia psilotachya*) is apparently only a local cause of hay fever. Bermuda grass is important in that region, but he especially stresses the importance of the false ragweeds. He says that patients who are sensitive to ragweed but have never been exposed to false ragweed react clinically to the latter almost immediately. Sometimes, however, it may take as long as three years.

Balyeat<sup>45</sup> finds that the Western water hemp (*acnida tamariscina*) is a very important factor as a cause of hay fever and asthma in Oklahoma. As a result of data collected from replies to questionnaires sent to professors of botany of colleges of several states he reports that there is a great prevalence of *acnida tamariscina* in Oklahoma, eastern and northern parts of Texas, eastern Nebraska, southern part of South Dakota, Iowa, and southern Michigan. There is a lesser prevalence of this plant in Illinois, Missouri, Arkansas, and Kentucky; there is a small amount in Ohio and New York. From these reports also it is shown that the closely related plant *acnida tuberculata* is prominent in Iowa, Wisconsin, Minnesota, and southern Michigan. Balyeat states that in Oklahoma he finds the Western water hemp responsible as a sole cause in 36 per cent and as a definite contributing cause in 35 per cent of the hay fever cases.

Scheppegrell<sup>46</sup> made inquiry from public officials of Mexico, and various sections of Central and South America in regard to the prevalence of hay fever. The Mexican government and several states in South America reported that there is practically no hay fever there while several states in Central America absolutely denied the existence of hay fever in their localities. It was Scheppegrell's opinion that hay fever in most of these countries is prob-

ably uncommon and also little understood. In Argentina Van Boeckel<sup>48</sup> reports that the early summer type of hay fever exists there and is apparently due to the grasses. They have also apparently a free season.

There are several rather meager reports concerning the botany of hay fever in European countries. Hansen<sup>49</sup> of Heidelberg finds grasses to be the predominating cause in Germany. Von Banzky<sup>50</sup> thinks that there is not much less hay fever in Germany than in America. The German hay fever season occurs chiefly during May and June and is due principally to timothy, but also to other grasses such as orchard grass, red fescue, June grass, etc. and to grains, rye, wheat and oats. The early spring and fall types are due to flowers, he thinks. In Denmark hay fever was studied by Baagø<sup>51</sup> with local pollens. Although the number of his cases is small he has been able to arrive at rather definite conclusions. He found that all of his cases reacted to the six grasses timothy, orchard grass, June grass, wild oat, ray grass, and meadow foxtail. All reacted to corn pollen. There were no reactions to wheat, oat or barley pollen. The tree pollens gave only weak and occasional reactions. He thinks that some of the fall cases are due to the artemisiae. The most important hay fever season is from the first of June to the middle of July. Delker reports two cases of hay fever in winter due to the Alpine grass pollen in the mattress. Both cases showed positive skin tests and were relieved by the removal of the mattresses.

In a preliminary communication Pirie<sup>52</sup> reports on the hay fever situation in South Africa. The grasses seem to be the most important factor, but none of the timothy family (genus *phleum*) is present. He considers the compositae also important because they are so numerous that close contact cannot be avoided. A rather peculiar cause of hay fever in that part of the world is the pollen of the pepper tree (*Schinus molle*). This tree is insect pollinated but under special conditions such as a dry high wind, and because of the great numbers of these trees used for street and road decorations in many localities it may cause considerable hay fever. From the same region Jurriaanse<sup>53</sup> reports two cases of hay fever treated with mixed extracts of the pollen of compositae, artemisiae and pepper tree with good results.

#### POLLEN STUDIES AND COUNTS

Studies in morphology and counts of atmospheric pollen have taken great strides in the last two years. Durham,<sup>54</sup> with the aid of data obtained from several allergists has reported comparative ragweed pollen records for nine large cities. It is interesting to note what enormous differences exist in the pollen concentration of the air of these cities. Oklahoma City with a maximum count of 3600 ragweed pollen per cubic yard, is at the top of the list. Next in order are Indianapolis, Kansas City and Chicago the latter having a maximum of 250 (in 1925). New Orleans and New York are next in order the latter having a maximum of 100. The cities having the lowest counts are Philadelphia, Richmond 12, and Oakland 6.

Rowe<sup>55</sup> has made the first report in which pollen counts were recorded for an entire year. On the east shore of San Francisco Bay he shows that pollen is present in the air practically every day throughout the year, except on

the days it rains continuously. In Rowe's articles are to be found drawings of the California pollen and key tables for their morphologic identification.

Duke and Durham<sup>57</sup> made comparison of variation of pollen content of the air from day to day in different localities in Kansas City, Missouri. They conclude that the variation of the severity in the patient's symptoms are explainable entirely by the variation in pollen count. Comparison of these pollen counts with those made in other cities, and those in other seasons, also offers an explanation to these authors why a given method of treatment may be successful in one city, or one year, and be almost a total failure in another city or in the same city in another season.

The factors which determine the pollen content of the air are analyzed by Balyeat<sup>58</sup>. He considers, of course, the abundance of plant life as the most important factor in determining the amount of pollen. Wind causes greater pollen counts and increases hay fever symptoms. Greatest pollination occurs during the morning hours. Rain frees the air of pollen, but subsequent sunshine may entirely overcome that factor. Before pollination rain helps weeds to grow larger and to produce more pollen.

Duke<sup>9</sup> determined the pollen content of air under various conditions of stillness. In a room kept closed for one and one-half years no pollen was found in the air. In a room closed for one day there were 3 pollen per cubic yard, in a room closed for one day with a fan going for twelve hours, 8 pollen, and in ventilated rooms on the same day, 20 pollen. The outside air count at the same time was 191. Experiments near the propeller of an aeroplane show pollen counts to be 100 or more than of still outside air. From these observations the author concludes that still rooms can be utilized for pollen-sensitive patients on bad days or nights, and that motor rides, train trips, and aviation should be avoided.

Identification of pollen by structural features are discussed at length by Piness and McMinn<sup>60</sup>. They give a complete key to pollen identification of California pollen but they emphasize the following points. Most grasses have pollen grains resembling grains of half-ripe field corn. In the compositae group the artemisiae pollen have elliptical form with furrows. The ambrosiacea are oval with definite spicules. All wind-borne spiculated pollens belonged to the ambrosiacea. The chenopodaciae (goosefoot family) have spherical pollen with round surface concavities. An abstract cannot possibly do justice to this article, and in order to study this subject the reader is urged to see the original publication, in which there are many microphotographs, tables, etc. In a very interesting but technical paper Wodehouse<sup>61</sup> attempts to demonstrate how a study of the structural character of pollen grains may be utilized in the identification of plants and their classification and phylogenesis. In this article he discusses chiefly the ambrosiacea pollen which he says can be recognized by their tricolpate character, short spines, and short furrows.

#### CHEMISTRY OF POLLEN

Whether or not the active principle of pollen is protein in nature is still a disputed question. Mell<sup>62</sup> of the University of Padua claims to have demonstrated that the active portion of pollen is protein, is undialyzable, becomes

inactivated by the action of proteolytic ferments, is destroyed by precipitating it with dealbuminizing substances, and provokes a positive Abderhalden reaction. These conclusions were arrived at by digestion experiments with pepsin, trypsin, trypsin, enterokinase and papain.

Attempts to separate the individual proteins of pollen have been made by Bernton, Jones and Csouky.<sup>64</sup> From timothy pollen they have isolated four active proteins, which they have designated proteose A, proteose B, albumin, and glutelin. Of 19 grass pollen cases 63 per cent showed cutaneous sensitiveness to two or more proteins, 21 per cent to proteose A, and 15 per cent to albumin only. The albumin constitutes one ninth of the total extractable protein content. The albumin sensitive patients have failed to derive benefit by treatment with extract of whole pollen, but were relieved by treatment with albumin fractions. Untreated albumin sensitive patients had no asthma. Proteose sensitive patients showed 72 per cent incidence of asthma. These workers made similar analyses of ragweed pollen and obtained proteose and albumin fractions. In 50 per cent of these there was skin sensitiveness to proteose and albumin, in 35 per cent to proteose alone, and in 14 per cent to albumin alone.

Allen and Lamson<sup>65</sup> have attempted to obtain purified pollen extracts by washing the pollen with 80 per cent alcohol and then following immediately by glycerol saline extraction. This removed a considerable amount of the inactive substances but the resulting extract was not quite as strong as the unpurified extracts. However in three individuals this purified extract gave a strong reaction whereas they were negative to the crude extract.

Stier and Hollister<sup>66</sup> find that Russian thistle pollen extracts deteriorate rapidly. They found that the best extracting medium was 7 per cent sodium chloride with 46 per cent glycerin. They say that the glycerinated extracts are the only extracts of Russian thistle pollen which will retain their potency for a number of months.

Working with the pollen of orchard grass Loeb<sup>67</sup> precipitated an albumin fraction with 90 per cent alcohol. This precipitate was tested on 7 hay fever patients and found to give a good cutaneous reaction but somewhat weaker than the aqueous extract of whole pollen. The alcohol filtrate gave no reaction. Some of the alcohol precipitate was digested with 5 per cent trypsin and diluted. This digested alcohol precipitate gave no reaction in the hay fever patients. Loeb concludes that the active principle of pollen extract is an albumin.

#### SPECIFIC TREATMENT

That the results of specific therapy continue to be good is evidenced by the contents of almost every paper on hay fever. Wynn,<sup>68</sup> in an excellent historical review, describes the evolution of the modern method of treatment. Others such as Chamberlain<sup>69</sup> report small series of cases which exemplify the benefits conferred upon the hay fever sufferer by pollen treatment.

There are many reports analyzing the results of treatment by specific methods. All are more or less in agreement that the majority of pollen cases can be benefited. Thus Piness<sup>70</sup> says that in more than 90 per cent of



his cases there has been some degree of relief. Berntson<sup>70</sup> reports improvement in 94 per cent of his cases. In an analysis of 980 patients observed during the season of 1926 Vander Veen, Cooke, and Spain<sup>71</sup> find that 85 per cent received from 75 per cent to 100 per cent relief. These authors confirm the observations of other allergists that the pollen asthmatics have a greater percentage of complete relief (75 per cent) than the pollen eczemas.

Conway,<sup>36</sup> reporting on 30 cases of hay fever treated at Colorado Springs, finds that all were relieved and 27 were entirely free from symptoms. Foss<sup>41</sup> of Montana, in a series of 184 cases over a period of four years, obtained relief in about 90 per cent. Those obtaining complete relief, however, constituted only 10 per cent.

In the foreign literature one finds increasing enthusiasm in the results of specific therapy in hay fever. I have already referred to reports from Argentina, South Africa, Denmark, etc. Freeman<sup>2</sup> of England writes favorably of pollen desensitization. In Germany Von Bausky<sup>70</sup> and Stiebel<sup>73</sup> report satisfactory results with pollen treatment, the latter author obtaining complete relief in 21 out of 40 cases. Loeb and Petow<sup>74</sup> comment upon the fact that specific desensitization is used very little in Germany. In 29 patients treated in various ways, with mixed grass pollens, single grass pollens, and peptone, the best results were obtained with mixed grass pollens. Harsen<sup>40</sup> reports 85 per cent of patients improved in a series of 23 cases treated by him. From Denmark comes additional endorsement of the efficacy of specific therapy in an article by Schwartz.<sup>75</sup> On the other hand, Hanhart of Zurich,<sup>76</sup> basing his experience on 3 cases of hay fever, reports discouraging results with pollen extracts.

*Causes of Failure*—While the results of pollen therapy as a whole are good there are many cases in which there is complete failure, and this may constitute the majority in the hands of men who have had little experience with this form of therapy. I have called attention to the fact that although with present methods of treatment one cannot produce relief in one hundred per cent of sufferers, nevertheless, attention to certain factors will lead to maximum results. These factors are the early preseasonal treatment continued into the season, the individualization of dosages of pollen and intervals of treatment according to the patient's reaction, the careful determination of the species of pollen to be used, and finally, the preservation of the potency of the pollen extract by refrigeration in concentrated form.

Cohen, Reicher, and Breitbart<sup>78</sup> express similar views. They say that causes of failure in hay fever treatment are due to incorrect diagnosis, inadequate or improper treatment due either to impotent extracts or insufficient number of injections or inadequate final dosage, the inability to develop tolerance and contact with an unusually large dose of pollen.

*Curability*—Very little has been said in the past about the curability of hay fever. However, as time passes and the number of cases treated thoroughly and repeatedly for many seasons is accumulating, we are coming to the realization that it is possible for hay fever to be permanently relieved. Thus, Henry<sup>79</sup> says that successful desensitization for 3 to 5 seasons frequently gives permanent immunity. Walker<sup>80</sup> analyzes a series of 100 "ap-

parently cured" have fever cases. He defines the word 'cure' as freedom from symptoms for two or more years without treatment. Of this number 78 have gone three or more years without treatment. These patients were treated at weekly intervals for a period of thirteen to sixteen weeks previous to the pollen season. In 22 cases the pollen tests have become negative, in 22 they were very doubtful when treatment was discontinued, in 12 cases there was marked change in the reaction and in 44 no marked change occurred. Waller's tables seem to show that the average duration of treatment in these cured cases is three and one half seasons with a minimum of 1 and a maximum of 6, and that after two seasons the patient is usually free from symptoms (in those that go on to ultimate cure). He concludes that when pollen tests become negative it seems to be safe to stop treatment in those in whom tests do not become negative it is desirable that the patient should have at least one season and preferably two of complete freedom from hay fever while being treated before treatment is discontinued.

*Constitutional Reactions*—The greatest barrier perhaps to the acceptance of specific pollen therapy by the general medical profession and the public has been the risk of constitutional reactions. Vander Veer et al.<sup>11</sup> find that in a series of 980 cases treated 10 per cent had constitutional reactions at some time during their course of treatment while these reactions occurred in one per cent of all injections. They also found that these reactions are more apt to occur toward the end of a course of treatment. They blame overdosage as the most important factor and also mention two other causes accidental injection into a venule and the change from an old to a new extract during the course of treatment. Bernton<sup>12</sup> states that constitutional reactions are due to overdosage of pollen extract or accidental intravenous injection. To avoid the latter he withdraws the needle partially after insertion and changes the axis slightly. To avoid serious reactions he advises two precautions: to have adrenaline at hand and to have the patient wait for fifteen to twenty minutes after pollen injections. To the above the reviewer would like to add that intramuscular injections which are frequently given with intent of subcutaneous administration and intraarticular injections further increase the possibility of constitutional reactions.

Kahn<sup>13</sup> calls attention to the fact that dosages and methods of administration of pollen extracts in children do not differ from those in adults.

The controversy of the relation of the two important ragweed pollens short and giant ragweed has been opened again. Aaron Brown<sup>9</sup> finds that treatment with one pollen protects against both pollens as well as treatment with both. On the other hand, treatment with ragweed pollen did not protect in the slightest degree against grass pollen<sup>6</sup> as indicated by natural exposure during the season or reaction to subcutaneous injection. He concludes that only one variety of ragweed pollen is necessary for the treatment of ragweed cases, but that the grass pollen must be used for grass cases.

*Perennial Treatment*—One of the most promising advances in specific therapy is the development of a method of treatment whereby the patient is treated all year round. This plan of treatment as described by Aaron Brown<sup>13, 14</sup> consists of the injection of pollen at three to four week intervals,

instead of the usual preseasonal short interval method. However, this should only be tried after the individual has been worked up to a large dose by the usual method. In addition to the fact that possibly the therapeutic results may be better Brown cites the advantages that treatment may be started at any time of the year and the actual number of treatments may be lessened. Kahn<sup>85</sup> has independently described a somewhat similar plan. He states that to maintain immunity to pollen he gives a maximum dose every week, sometimes as long an interval as three weeks, and given indefinitely. He says that this method can be modified by holding the protection by a weekly dose of one-tenth or one-fourth of the maximum protective dose and running up the treatment a few weeks before the onset of the following season. Vander Veer et al<sup>71</sup> also describe the perennial method of treatment which they say they have used since 1920, but have used it on a larger scale since 1925. They give monthly injections of the maximum dosage attained during the season. They claim that their results are fully as satisfactory as by the preseasonal method and that it aims at permanent immunity.

*Oral Treatment*—That pollen extracts may produce immunity when given orally has been demonstrated by Black<sup>86, 87</sup>. His method consists of giving orally, three times daily, a 5 per cent extract of pollen in a glass of water or milk. The initial dose is 10 drops and every dose is increased by 10 drops until 60-drop doses are taken. The administration is begun with the first appearance of hay fever symptoms. In a comparison of 73 orally treated with 61 hypodermically treated patients he finds that oral administration has many advantages although protection is not secured as frequently. Stiebel<sup>73</sup> combines injections with oral administration. The latter consists of giving four times daily 50 drops of his special pollen extract "Pollisatin" in a half glass of water.

*Pollen-Free Atmosphere*—The benefit derived from residence in pollen-free resorts is too well-established to allow of any dispute. Cohen<sup>88, 89, 90</sup> has continued his observations in obtaining such a pollen-free atmosphere at home. By means of a motor-driven suction fan which sucks in air through a window opening and filters it through layers of felt a pollen-free room can be obtained. In such a room a pollen-sensitive patient may remain free from symptoms, or be completely relieved of his symptoms in two to seven days' residence if his manifestations are well established. To keep a patient comfortable the use of such a pollen-free atmosphere from eight to twenty-two out of every twenty-four hours will be required, depending on the individual's tolerance.

#### EPINEPHRIN, EPHEDRIN, AND SIMILAR DRUGS

Balyeat<sup>91</sup> finds epinephrin useful in simultaneous injection of pollen extracts in cases which have a tendency to systemic reactions. He admonishes, however, that in such cases adrenalin must be continued in subsequent injections of the pollen.

Ephedrin, the alkaloid obtained from the Chinese plant Ma Huang, has continued to demonstrate its sphere of usefulness in hay fever as well as in asthma. There are several papers dealing specifically with the use and results of ephedrin therapy, there are also many other papers dealing with other fea-

tures of hay fever or asthma in which the use of ephedrin is alluded to. It has been found possible to obtain results in either of two ways: locally, by spraying or applying solutions to the mucous membranes; and systemically, by oral administration.

Wilkinson<sup>9</sup> reports the use of ephedrin orally in 5 cases of hay fever with good results. Leopold and Miller<sup>13</sup> obtained relief in 5 out of 11 cases treated. Althausen and Schumacher<sup>14</sup> reporting the results in 62 cases of hay fever and asthma, claim benefit in 80 per cent of their cases. Gaarde and Maytum<sup>15</sup> describe the results obtained on 36 cases of autumnal hay fever treated with ephedrin, orally, locally or combined. Of this series only 11 had had pre-seasonal pollen treatment. Ephedrin orally (25 mg) gave almost complete relief lasting four or more hours in 54 per cent of the cases and partial relief in 16 per cent. In 10 cases of pollen asthma 5 were relieved and 3 only moderately relieved. The use of a 3 per cent ephedrin spray two or three times daily resulted in marked relief in 28 per cent of the cases and only fair results in 48 per cent. No relief was obtained in the asthma cases by the latter method. Piness and Miller<sup>6</sup> obtained relief in only one case out of 5 treated orally, and 18 out of 20 treated locally. Green<sup>18</sup> reports good results from local application of ephedrin in 2 cases of hay fever. He tried it in only one case by the oral method with no relief.

Balveat<sup>19</sup> suggests a new use for ephedrin: its employment prior to injection of pollen extract to prevent constitutional reactions. For this purpose he advises the patient to take 35 mg ephedrin orally fifteen minutes before the injection of pollen or 50 mg when the reaction begins.

The use of ephedrin is limited considerably by its frequent production of undesirable symptoms. The most constant effects as mentioned by most authors,<sup>9, 13, 14, 15, 16, 17, 18</sup> are palpitation, tremor, nervousness, insomnia and nausea. There are also described by others such effects as weakness, vomiting, perspiration and a sense of constriction around the head. Althausen and Schumacher<sup>14</sup> have obtained undesirable effects in 30 per cent of their cases and in 12 per cent so severe that the therapy had to be discontinued. Gaarde and Maytum<sup>15</sup> found such marked toxic effects in 16 per cent of their cases as to make its use in those instances impossible. Piness and Miller<sup>17</sup> report untoward results in at least 20 per cent of their cases. Balveat<sup>19</sup> states that in 20 per cent of his cases (hay fever and asthma) the symptoms were so severe that the drug was discontinued.

From the above observations and from my own experience with ephedrin therapy I am of the opinion that: (1) ephedrin has some field of usefulness as an adjunct in the treatment of hay fever; (2) the results are more evident in the cases that are treated by pre-seasonal pollen injections and may be negligible in untreated cases; (3) its usefulness is greatly limited by its tendency to produce undesirable symptoms; (4) locally it has some beneficial but rather short-lived effects; (5) it has in no way changed the status of nor the necessity for, desensitization therapy.

Because of the undesirable symptoms and because of its rather high cost, attempts have been made to synthesize drugs similar to ephedrin. Kreitmar<sup>100</sup> reports experimental findings with the use of a new drug which he calls

"ephetonin" Effects on blood pressure of normal and decerebrated cats, on the heart, respiration, bronchial musculature, and vagina uterus of guinea pig were the same with ephetonin as with ephedrin. Fischer<sup>101</sup> says that ephetonin is chemically identical with ephedrin, except that the former is optically inactive. He states further that the pharmacologic action is the same, but it has the advantage in that it is cheaper. Berger and Ebster<sup>102</sup> obtained remarkable results by spraying the nose and eyes of hay fever cases with 5 per cent ephetonin. Their conclusion with respect to the oral use of ephetonin (in asthma) are that its action is slower than ephedrin, that equal doses produce less effects, and that there are cases benefited by ephedrin and not by ephetonin. They claim the following advantages for ephetonin: that undesirable effects are much less, that it is cheaper, and that it may be used as substitution therapy in cases of tolerance to adrenalin or ephedrin.

Miller and Piness<sup>103</sup> report a new synthetic substitute for ephedrin. It is the sulphate of phenolethanolamine. Orally it has only a slight effect on hay fever or asthma, but locally, in 2 per cent solution it has as good an effect as 3 per cent ephedrin.

#### OTHER MEDICINAL TREATMENT

A number of reports deal enthusiastically with the use of various drugs in hay fever. Sterling<sup>14</sup> recommends the use of calcium and phosphorus, especially the latter, and states "since our administration of phosphorus in addition to desensitization in hay fever patients the results have been almost 100 per cent freedom from symptoms."

Nussbaum<sup>104</sup> of Germany used "phocajod," an iodine preparation, on himself and several other cases with good results. Hamburger<sup>105</sup> recommends the bromides very highly. He says that one gram of sodium bromide mornings and evenings and one-half hour prior to going out doors will keep most cases free from symptoms. He says further that the effect may last a few hours to two or three days. Nitrohydrochloric acid is strongly recommended by Beckmann,<sup>106-107</sup> basing his experience on 17 cases of hay fever including himself. He claims to have obtained 100 per cent relief in all of these patients by the administration of about 10 minims of nitrohydrochloric acid in two-thirds of a glass of water after each meal and upon retiring.

Some of foreign literature contains mention of modes of therapy which to the American observer seem very strange and fantastic. Thus, Bonjour<sup>108</sup> recommends morphine in small doses basing his contentions on his use of it in the past thirty-five years. Proby,<sup>109</sup> among other remedies, speaks of the use of a hydroalcoholic ether extract of jaborandi. Billard<sup>110</sup> recommends the mineral waters of Mont-Dore on the supposition that hay fever is due to a change in the  $P_H$  concentration of the nasal reactions. His explanation is as follows: Each pollen and each female flower have their own  $P_H$  reaction. Fecundation can only result when the pollen of certain  $P_H$  reaction come in contact with the ovum. If the pollen of the wrong  $P_H$  concentration reaches the ovum no fecundation takes place. He regards the contact of pollen with the mucous membranes of the eye, nose, etc., as "monstrous fecundation." Whether or not clinical symptoms will result in a particular individual de-

depends on the  $P_H$  reaction of his nasal secretions. Usually a too high  $P_H$  causes a breaking up of pollen and a toxic reaction. He therefore claims to have obtained very remarkable results with the use of mineral waters in hay fever.

Hirsch and Loewe<sup>111</sup> describe a new drug, the lactone of gallic acid ethanolaninochlorhydrate which they call salyamm. This drug is chemically allied to adrenalin, is water and alcohol soluble, occurs in colorless crystals, and has about one hundredth the toxicity of adrenalin. They claim to have produced remarkable results in 50 hay fever cases by the use of this drug in doses of two to six tablets of three fourths grains daily. Failure occurred in only two instances. These patients were able to go in fields, take auto rides, train rides, etc. Hirsch<sup>11</sup> further says that in the administration of this drug to 1000 individuals he has found it absolutely nontoxic. In another communication<sup>112</sup> he stresses the fact that no undesirable effects are produced by this drug, and again relates his experience with the 50 hay fever cases previously reported on. He says, however, that these cases were treated during the 1925 and 1926 seasons, which he admits were very mild seasons. Another report on this drug comes from Gerlach<sup>114</sup> who cites the good effects produced on himself, a hay fever sufferer for eighteen years.

Spieß<sup>11</sup> thinks that hay fever is a disturbance of the balance of internal secretions. On the basis of the fact that dermatologists had obtained excellent results with thymus extract in psoriasis and other skin lesions and that many of asthmatics had had a previous skin disturbance, it occurred to him that thymus treatment might be of value. He cites his success in several cases of asthma and hay fever. In hay fever he begins therapy with the onset of the first sneezing and gives one to two ampoules of thymophorin intramuscularly daily for 10 to 15 doses. He also suggests the use of calcium in addition and also stimulation radiation of the thymus.

#### NONSPECIFIC AND LOCAL THERAPY

The French school seems to be fond of nonspecific methods of treatment of hay fever. Thus Vallery Radot and Grouet<sup>11</sup> employ intradermal injections of 50 per cent solution of de Witte's peptone. They begin on the second or third day of the symptoms and give daily injections for twenty days and notice results mostly on the last injection. The dose is 0.1 cc with daily increments of 0.1 cc. Flaudin<sup>117</sup> believes better results are obtained with intradermal autoserotherapy and after fourteen years' experience with this method he is convinced that that is the best mode of treating hay fever patients.

Duke<sup>10</sup> Huiwitz<sup>118</sup> and others agree that nasal surgery must be done with distinct fear in a case of hay fever or asthma. Nevertheless, according to Huiwitz, and observations by others, there are times when nasal infections may be a predisposing or aggravating cause of hay fever. In such proper cases it may be considered advisable to do some local surgery.

Leich<sup>1</sup> recommends some kind of local treatment which he calls "filtration method." Just what the nature of his treatment is seems quite in doubt from the contents of the article. Hollender and Cottle<sup>119</sup> in discussing the treatment of hay fever and asthma, advise the substitution of morphine for adrenalin, recommend the use of ultra violet therapy by general body expo-

sue and intranasal application, use calcium and thyroid orally, and employ nasal tamponage for one half hour with aigylol Kagan<sup>120</sup> speaks of "detoxication," relieving body and nose abnormalities, Dowling tamponage, ultra-violet exposures, x-ray treatment (for asthma), but admits that "a further treatment when indicated is pollen extracts as a preventative and curative measure

Hamm<sup>1-1</sup> cites the cure of hay fever in 20 cases by diathermy with special nasal applicators A and L Pokorny<sup>122</sup> cite two cases of hay fever in physicians who obtained remarkable results with x-ray therapy directed to the root of the nose

While practically all of the above nonspecific methods of treating the hay fever patient are unproved, uncorroborated and unconvincing, there are some encouraging results reported by American workers with vaccine therapy These men are careful and experienced hay fever specialists, modest in their claims and cautious in their assertions Thus, Grafton Tyler Brown<sup>34</sup> says that "cold" vaccines are a valuable adjunct to pollen therapy in coseasonal treatment Scheppegeiell and Thibeige<sup>123</sup> analyze the effects of vaccine therapy in a series of 1399 hay fever patients They used vaccines only during the season and gave it every other day They conclude that vaccines used in this manner increase the percentage of good results Three types of vaccines were employed (1) the usual mixed catarrhal-respiratory vaccine, used in cases in which the acute attack seems to be due to infection, (2) vaccine consisting of organisms based on bacterial tests of a large number of hay fever cases during acute attacks, and employed in cases in which symptoms appeared to be due to increased pollen infestation, (3) and autogenous vaccines, used in cases which did not respond to stock vaccines

As in reports on therapy in other fields, some of the literature on hay fever therapy is rather confusing from the standpoint of its evaluation because each writer reports his own results by his own particular method without great attempt at personal comparison with other methods Especially in specific pollen therapy there are many variations in technique, preseasonal and coseasonal, early preseasonal intensive method, long-interval method, large and small maximum doses, adjuncts such as vaccine therapy, light therapy, etc Ramirez,<sup>124</sup> in the most illuminating clinical paper in the last two years, has attempted to crystallize all of these questions in one report He made a statistical analysis of 425 cases of hay fever treated by him from 1919 to 1927 and classified them into various classes, groups, subgroups, series, etc, according to variations in methods of treatment Each question is answered by the results of treatment in an individual series of cases From these studies the author draws the following conclusions "It is evident that the best results are obtained in cases coming about three months before the expected date of pollination, receiving two weeks intensive treatment, increasing the dose of pollen rapidly and continuing injections up to the pollen season, reaching as high a dose as possible then continuing injections of pollen twice a week throughout the entire season in addition, injections of autogenous vaccines prepared from the nose, and local treatment to the nose and eyes with increasing concentrations of pollen solutions, preseasonal and seasonal "

## REFERENCES

- 1 Duke W W Advancement in Knowledge of Allergies Related to Otolaryngology, Arch Otolaryng 6 440 1927
- 2 Duke, W W Advance in the Field of Allergies Related to Otolaryngology During the Years 1927 and 1928 Arch Otolaryng 8 573 November, 1928
- 3 Eggeston, A A Practical Suggestions for Nurses Handling Hay Fever Patients Trained Nurse 78 610 1927
- 4 Clarke J A Jr Donnally H H and Coca A F Studies in Specific Hypersensitiveness XXXII On the Influence of Heredity in Atopy J Immunol 15 9 1928
- 5 Balyeat R M Acquisition of Specific Hypersensitiveness Based on a Study of 1000 Asthma and Hay Fever Cases South M J 21 554 1928
- 6 Baldwin, L B Skin and Mucous Membrane Reactions in Hay Fever J Immunol 13 345 1927
- 7 Bristow W J The Ocular and Rhinological Manifestations of Allergy J South Carolina M A 24 280 1928
- 8 Vaughan, W T Pollinosis Constitutional and Local Factors Arch Int Med 40 386, 1927
- 9 Balyeat R M The Importance of Orris Root as an Etiologic Factor in Hay Fever and Asthma, J LAB & CLIN MED 13 516 1928
- 10 Duke W W Allergy as Related to Otolaryngology Ann Otol Rhin and Laryng 36 830 1927
- 11 Eyerman C H Nasal Manifestations of Allergy Ann Otol Rhin and Laryng 36 808 1927
- 12 Brown G T Eosinophilia in Asthma, Hay Fever and Allied Conditions J LAB & CLIN MED 12 1145 1927
- 13 Sternborg, L Eosinophilia in Hay Fever Am J M Sc 176 82 1928
- 14 Sterling, A Value of Phosphorus and Calcium in Asthma Hay Fever and Allied Diseases J LAB & CLIN MED 13 997 1928
- 15 Leach E S Hay Fever, Its Cause Pathology and Treatment West M Rev 13 7-7 1928
- 16 Byrd H Pathological Currents and Hay Fever Am Med 23 169 1928
- 17 Kahn, I S Pollen Toxemia in Children J A M A 88 241 1927
- 18 Kahn I S Pollen Hay Fever and Asthma in Children, Texas State M J 23 209, 1927
- 19 Kahn I S Essential Differences in Chronic Pollen Hay Fever and Asthma in Children and in Adults J LAB & CLIN MED 12 1197, 1927
- 20 Lamson, R W, and Alles G Evidence of the Specificity of the Intracutaneous Pollen Test in Man J LAB & CLIN MED 13 1149 1928
- 21 Hurwitz S H Asthma and Hay Fever California & West Med 29 2 1928
- 22 Kahn, I S Significance of Negative Skin Tests in Pollen Hay Fever and Asthma in Infants and Young Children Treatment South M J 21 559 1928
- 23 Kahn I S and Grothaus Emma M Incidence and Significance of Negative Skin Tests in Pollen Asthma in Infants and Young Children, J LAB & CLIN MED 13 949 1928
- 24 Feinberg S M The Nonspecific Diagnosis of Allergic Disease Further Observations, J LAB & CLIN MED 13 947 1928
- 25 Lamson R W and Miller H Pollen Allergy Genesis of Skin Hypersensitive ness in Man Arch Int Med 40 618 1927
- 26 Lamson R W Pinness G and Miller H Pollen Allergy with Special Reference to Skin Reactions before and after Treatment with Fall Pollens Am J M Sc 176 799 1928
- 27 Lamson R W Pinness G and Miller H Pollen Allergy Skin Reactions of Patient before and after Treatment with Spring (Grass) Pollens Am J Med Sc 176 791 1928
- 28 Brown A Studies in Hypersensitiveness on the Question of the Identity of the Atopens of the Pollens of High Ragweed and Low Ragweed J Immunol 13 73 1927
- 29 Thomsen O, and Baage K Experimentelle Untersuchungen über die Antigene Wirkung Verschiedener Pollenarten Acta Med Scand (Supp No 26) p 21 1928
- 30 Thomsen O and Baage K Experimentelle undersøgelse over den Antigene Virkning af Forskellige Pollenarter Ugeskr f Laeger 89 1171 1927
- 31 Loeb L F Über die Sensibilisierung mit Allergen und über die Natur der Allergene Klin Wehnsehr 7 803 1928
- 32 Grove Ellen F Studies in Specific Hypersensitiveness XXXI On Passive Transfer of Atopic Hypersensitiveness to Monkeys J Immunol 15 3 1928
- 33 Black J H Relation of Allergic Reagin to True Atopen in Pollen South M J 21 373, 1928



- 34 Brown, G T Late Spring or Summer Hay Fever in District of Columbia and Vicinity, *Ann Int Med* 1 169, 1927
- 35 Bernton, H S Hay Fever Caused by Pollen of Paper Mulberry (*Papirus Papyrus* Kuntze), *J LAB & CLIN MED* 13 829, 1928
- 36 Conway, L A Hay Fever, *Colorado Med* 25 266, 1928
- 37 Waring J, and Pope, M Cotton of Cottonwood Tree, a Factor in Hay Fever in Preliminary Report, *Colorado Med* 34 218, 1927
- 38 Chamberlain, C T Hay Fever Treatment in Pacific Northwest, *Ann Otol, Rhin, and Laryng* 36 1083, 1927
- 39 Rowe, A H A Study of the Atmospheric Pollen and Botanic Flora of the East Shore of San Francisco Bay, *J LAB & CLIN MED* 13 416, 1928
- 40 Stewart, Z W Hay Fever, *Med Woman's J* 34 162, 1927
- 41 Foss, A R Hay Fever in Montana, *Minnesota Med* 10 587, 1927
- 42 Albert, H Hay Fever in Nevada, California & West Med 27 65 1927
- 43 Balveat, R M Seasonal Hay Fever, a Study of 300 Consecutive Cases, *J Oklahoma M A* 21 106, 1928
- 44 Spitz, H Pollen Sensitization, *J Tennessee M A* 20 78, 1927
- 45 Phillips, W W Ragweed Hay Fever in Salt River Valley, *Southwest Med* 12 49, 1928
- 46 Balveat R M, and Stemen, T R Hay Fever and Asthma, Distribution and Importance of Acacias as a Cause of Hay Fever and Asthma in United States, *Am J M Sc* 174 639, 1927
- 47 Scheppegegrell, W Hay Fever in Mexico, Central and South America *M J & Rec* 128 105, 1928
- 48 Van Boeckel, L Allergic States in General and Hay Fever in Particular, *Revue sud am de endocrinol* 10 170, 1927
- 49 Hansen, K Die Kausaltherapie des Heufiebers durch Desensibilisierung, *Deutsche med Wchnschr* 54 1447, 1928
- 50 von Bauszky, L Somatische Betrachtungen über Diagnose und Desensibilisierende, Therapie des Heufiebers, *Med Klin* 24 1086, 1928
- 51 Baage, K H Einige Untersuchungen über Kutanreaktionen bei Heufieber, *Acta Med Scand* 67 297 1927
- 52 Baage, K H Einige Untersuchungen über Kutanreaktionen *Acta med Scandinav* (supp No 26), p 19, 1928
- 53 Baage, K H Nogle Undersøgelser over Kutanreaktioner ved Høfeber, *Ugeskr f Læger* 89 1167, 1927
- 54 Pirie, J H H Hay Fever in South Africa, Its Causes and Treatment, a Preliminary Communication, *J M A South Africa* 2 374, 1928
- 55 Turrianse, A Hay Fever and Other Allergic Conditions, *J M A South Africa* 2 388, 1928
- 56 Durham, O C Comparative Ragweed Records for Nine Large Cities, *J LAB & CLIN MED* 13 967, 1928
- 57 Duke, W W, and Durham, O C Pollen Content of the Air, Relationship to the Symptoms of Hay Fever, Asthma and Eczema, *J A M A* 90 1529, 1928
- 58 Balveat, R M Factors which Determine the Pollen Content of the Air, *J LAB & CLIN MED* 12 1151, 1927
- 59 Duke, W W The Pollen Content of Still Air in Relationship to Symptoms and Treatment of Hay Fever, *J A M A* 91 1709, 1928
- 60 Piness, G, and McMill H E The Role of the Structural Features of Pollen Grains in Identifying the Most Important Hay Fever Plants of California, *J LAB & CLIN MED* 12 1164, 1927
- 61 Wodehouse, R P Pollen Grains in the Identification and Classification of Plants I The Ambrosiaceae, *Bull of the Torrey Botanical Club* 55 181, 1928
- 62 DeKler, H Aphorismen zur Asthmi-frage Heufieber im Bett—Taubenfedern—und Bienenstiehasthm, *München med Wchnschr* 75 1123, 1928
- 63 Melli G Sulla Natura delle Sostanze Sensibilizzanti dei Pollini, *Riforma med* 43 511, 1927
- 64 Bernton, H S Jones, D B, and Csonka, F A Pollen Proteins and Their Clinical Significance in Hay Fever, Preliminary Communication, *South M J* 20 257, 1927
- 65 Alles, G A and Lamson, R W The Preparation and Purification of Pollen Extracts *J Immunol* 14 181, 1927
- 66 Stier, F E, and Hollister, G L A Comparative Study of Pollen Antigens as Determined by the Skin Reaction, *J LAB & CLIN MED* 12 1139, 1927
- 67 Loeb, L F Über die Natur der Allergene II Pollen vom Dactylis Glomerata (*Knauelgras*), *Klin Wchnschr* 7 1078, 1928
- 68 Winn, J Hay Fever, the Evolution of Modern Therapeutic Concepts, *J Indiana M A* 21 327, 1928
- 69 Piness, G Allergy of Respiratory Tract, *M J Australia* (supp 16) p 500, 1927
- 70 Bernton H S Pollinosis Wisconsin *M J* 26 242, 1927

- 71 Vander Veer A, Jr Cooke R A, and Spain W C Hay Fever Diagnosis Am J M Sc 174 101, 1927
- 72 Freeman J Treatment of Hay Fever Lancet 1 940 1927
- 73 Strebel, J Über Spezifische Behandlung de Heusiebers mit der Pollysatz methode Schweiz med Wehnschr 57 402 1927
- 74 Loeb L F and Petow H Erfahrungen über die Desensibilisierende Behandlung des Heusiebers Klin Wehnschr 6 57 1927
- 75 Schwartz, T To Tilfaelde af Heufeber Ugesk f Læger 99 323, 1928
- 76 Hannhart E Über das Heusieber und Seine Bekämpfung, Schweiz med Wehnschr 58 662, 1928
- 77 Feinberg S M Hay Fever Treatment Factors Lending to Maximum Results, Clin Med & Surg 35 390 1928
- 78 Cohen, M B Reicher, J and Breitbart J Causes of Failure in Hay Fever Treatment Ohio State M J 24 37, 1928
- 79 Henry J P The Diagnosis and Treatment of Hay Fever and Asthma J Tenne see M A 21 134, 1928
- 80 Walker I C One Hundred Cases of Apparently Cured Pollen Hay Fever J A M A 99 759 1928
- 81 Bernton H S Notes on Constitutional Reactions in Hay Fever Therapy J Lab & Clin Med 13 181 1927
- 82 Brown A On the Question of the Specificity of the Protective Effect of the Specific Treatment of Hay Fever J Immunol 15 391 1928
- 83 Brown A A New Plan for Applying the Specific Treatment of Pollen Hay Fever (Perennial Treatment) Preliminary Report J Immunol 13 273 1927
- 84 Brown A Perennial Treatment of Seasonal Hay Fever, Bull N Y Acad Med 3 536 1927
- 85 Kahn I S A Practical Method of Maintaining Induced Pollen Immunity, J Lab & Clin Med 12 77, 1927
- 86 Black, J H The Oral Administration of Pollen J Lab & Clin Med 12 1166, 1927
- 87 Black J H Oral Administration of Pollen Clinical Report J Lab & Clin Med 13 799 1928
- 88 Cohen M B The Prophylaxis and Treatment of Hay Fever and Asthma by Means of Pollen Filters, Clin Med & Surg 34 116 1927
- 89 Cohen, M B The Prophylaxis and Treatment of Hay Fever and Asthma in Rooms Made Pollen and Dust Free by Means of Mechanical Filters J Lab & Clin Med 13 59, 1927
- 90 Cohen, M B Further Observations on the Use of Filtered Air in the Diagnosis and Treatment of Allergic Conditions J Lab & Clin Med 13 963 1928
- 91 Bulyeat, R M Clinical Use of Epinephrin in Allergic Diseases J Lab & Clin Med 13 1019 1928
- 92 Wilkinson C R Ephedrin in Asthma and Allied Conditions Clinical Report J South Carolina M A 23 272 1927
- 93 Leopold, S S and Miller T G Use of Ephedrin in Bronchial Asthma and Hay Fever Its Effects in 59 Cases and in 11 Cases Respectively J A M A 88 1782 1927
- 94 Althausen, T L and Schumacher I C Ephedrin Therapy 62 Cases Arch Int Med 49 851 1927
- 95 Gaarde F W, and Maytum C K Further Observations on the Treatment of Hay Fever with Ephedrin, J Lab & Clin Med 12 1295 1927
- 96 Gaarde, F W and Maytum C K Further Observations on the Treatment of Hay Fever with Ephedrin Am J M Sc 174 835 1927
- 97 Piness G and Miller, H Ephedrin in Hay Fever and Asthma J A M A 89 515, 1927
- 98 Green R The Treatment of Asthma and Hay Fever with Ephedrin Preliminary Communication Malayan M J 3 10 1928
- 99 Balyeat R M Clinical Use of Ephedrin in the Treatment of Asthma and Hay Fever, J Oklahoma M A 20 3 1927
- 100 Kreitmar, H Ueber einen Synthetischen Körper mit der Wirkung des Naturheils Ephedrins (Ephetonin) München med Wehnschr 74 199 1927
- 101 Fischer W Ueber Ephetonin Merek, einen Synthetischen Körper von der Wirkung des Ephedrins München med Wehnschr 74 1947 1927
- 102 Berger W and Ebster, H Über Ephetonin München med Wehnschr 74 1087 1927
- 103 Miller H and Piness, G A Synthetic Substitute for Ephedrin The Chemistry Pharmacology and Clinical Application of Phenylethanolamine Sulphate J A M A 91 1033 1928
- 104 Nussbaum, R Neuasthma und Erfahrungen mit Phocajod Med Klin 23 1067 1927
- 105 Hamburger F Brom Gegen Heuschnupfen München med Wehnschr 74 802 1927
- 106 Beckman H Nitrohydrochloric Acid in Treatment of Hay Fever Am J M Sc 174 525, 1927

- 107 Beckman, H Nitrohydrochloric Acid in Hay Fever, Wisconsin M J 27 253, 1928
- 108 Bonjour, J Le Traitement Abortif des Rhumes et de Rhumes de Foins, Schweiz med Wehnschr 57 598, 1927
- 109 Probst, H Le Coryza Spasmodique, J de Méd de Lyon 8 445, 1927
- 110 Billard, G Considerations sur le pH des Fleurs, le Rhume des Foins, et les Cures Hydrominérales, Paris Méd 1 283, 1927
- 111 Hirsch, C and Loewc, S Salivamin Kahlbaum, seine Verwendung als Heuschnupfenmittel, nebst Pharmakologischen Vorbemerkungen, Deutsche med Wehnschr 53 791, 1927
- 112 Hirsch, C Zur Therapie des Heuschnupfens mit Salivamin Kahlbaum, Ztschr f Laryng Rhin 16 75, 1927
- 113 Hirsch, C Sulla terapia della corizza da fiene, Rassegna internaz di clin e terap 8 690, 1927
- 114 Gerlach, W Salivamin als Heuschnupfenmittel, Deutsche med Wehnschr 54 1255, 1928
- 115 Spiess, G Der Einfluss Endokriner Drusenextrakte, insbesondere des Thymusdruse auf Asthma, Heuschnupfen, u a Ztschr, f Hals Nasen u Ohrenh 18 250, 1927 28
- 116 Vallery Radot, P, and Giroud, P Survey of Recent Studies in Hay Fever, Paris Méd 1 151, 1928
- 117 Flandin, C Semeiology, Pathogenesis and Lines of Treatment in Hay Fever, Bull Méd Paris 42 665, 1928
- 118 Hurwitz, S H Indications for Surgery in Hay Fever, California & West Med 27 212, 1927
- 119 Hollender, A R, and Cottle, M H Survey of Progress in Hay Fever and Asthma Therapy, Clin Med & Surg 34 430, 1927
- 120 Kagan, S R Pathogenesis and Treatment of Hay Fever and Bronchial Asthma with Special Reference to Physical Therapy, Clin Med & Surg 34 526, 1927
- 121 Hamm Die Heilung des Heusiebers, Klin Wehnschr 6 574, 1927
- 122 Pokorny, A & L Die Rontgenbehandlung des Heuschnupfens, Strahlentherapie, 28 808, 1928
- 123 Scheppegegrell, W, and Thiberge, N F The Treatment of Hay Fever and Asthma, New Orleans M J 81 337, 1928
- 124 Ramirez, M A Statistical Report on the Treatment of Hay Fever, Am J M Sc 176 856, 1928
- 125 Huber, Harry L The Variability of Skin Reactions to Pollens, Read before the American Association for the Study of Allergy, Minneapolis, Minn, June 12, 1928

185 NO WABASH AVENUE

# LABORATORY METHODS

## A SUGGESTED MODIFICATION OF THE KRAMER TISDALL METHOD FOR THE MICROCHEMICAL ESTIMATION OF IONIZABLE CALCIUM IN BLOOD PLASMA\*

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**I**N VIEW of the wide use which is being made of the Kramer Tisdall<sup>1</sup> method for the determination of calcium in blood it has seemed appropriate to publish the results of a study, which has been made in these laboratories, of the original method and of later modifications by Tisdall<sup>2</sup> and by Clark and Collip<sup>3</sup>

Many attempts at recovery of calcium from a solution of known concentration using the Tisdall technic modified to the extent of one additional washing of the calcium oxalate precipitate, yielded irregular values

At first it was suspected that the low results obtained were due to the slight solubility of calcium oxalate in the 0.5 per cent  $\text{NH}_4\text{OH}$  (2 c.c. of  $\text{NH}_4\text{OH}$  sp. gr. 0.90 diluted to 100 c.c.) used in washing. Although this error was obviated by using 0.5 per cent  $\text{NH}_4\text{OH}$  previously saturated with calcium oxalate the results obtained were very irregular and still below the known value of the solution

Our next observation was that calcium oxalate precipitated from a solution of pure calcium chloride, although well packed after centrifugation was not matted as was the case with calcium oxalate precipitated from blood plasma

Subsequent experiments using the modified Tisdall technic demonstrated that added calcium could be quantitatively recovered from blood plasma notwithstanding the fact that its recovery from calcium solutions was uncertain. It is very probable that small amounts of fibrinogen and other protein are absorbed by the calcium oxalate and are thus responsible for the more adherent precipitate obtained from blood plasma rendering loss on decantation less likely

The best results at recovery of calcium from a known aqueous solution of calcium chloride were obtained when the original technic of Kramer Tisdall was followed, with the slight modification that 0.5 per cent ammonium hydrate saturated with calcium oxalate was used in washing. From the data in Table I, it is also evident that three washings are necessary to remove excess oxalate

If the precaution is taken of using a centrifuge tube of not more than 3 to 4 mm. in diameter at the tip, the fluid supernatant to the calcium oxalate

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TABLE I  
DOG PLASMA

NUMBER OF TIMES WASHED	NUMBER OF DETERMINATIONS	MEAN VALUE MG PER 100 CC OF PLASMA	MEAN VARIATION MG PER 100 CC OF PLASMA	KNOWN CALCIUM VALUE, MG PER 100 CC
1	4	10.66	$\pm 0.10$	10.00
2	4	10.25	$\pm 0.05$	10.00
3	3	10.13	$\pm 0.03$	10.00

TABLE II  
RABBIT PLASMA

SERIES	NUMBER OF TIMES WASHED	NUMBER OF DETERMINATIONS	MEAN VALUE MG PER 100 CC	MEAN VARIATION, MG PER 100 CC	SEPARATION OF WASH LIQUID
1	3	5	13.60	$\pm 0.10$	decantation
2	3	3	13.55	$\pm 0.05$	siphoned off
3	3	5	18.65	$\pm 0.15$	decantation

precipitated from serum or plasma may be removed by decantation (Tisdall's technique) equally as well as by the siphon method used by Kramer-Tisdall as is shown in Table II

In Series 3 of Table II, 5 mg. of calcium were added, and almost complete recovery realized

From Table III it is apparent that the solubility of calcium oxalate in dilute ammonia water is not a serious factor unless it is allowed to remain in contact with the precipitate for some time. Our proposed modification of using ammonium hydroxide previously saturated with calcium oxalate obviates any chance of loss by solubility, and renders the method more convenient, as the precipitated calcium oxalate may be left for any length of time in contact with the wash liquid

TABLE III  
DOG PLASMA

METHOD OF WASHING PRECIPITATE	NUMBER OF DETERMINATIONS	MEAN VALUE, MG PER 100 CC OF PLASMA	MEAN VARIATION, MG PER 100 CC OF PLASMA	WASH AMMONIA REMOVED BY DECANTATION
0.5% ammonia water	3	9.20	$\pm 0.05$	at once
0.5% ammonia water saturated with cal- cium oxalate	4	9.20	$\pm 0.10$	at once
0.5% ammonia water	"	8.90	$\pm 0.10$	24 hr later
0.5% ammonia water saturated with cal- cium oxalate	3	9.25	$\pm 0.10$	24 hr later

The modified Kramer-Tisdall method as herein described, when applied to plasma, separated from blood rendered noncoagulable by heparin, does not give the total calcium present, but only that which is in sufficiently ionizable form to be precipitated as the oxalate. In order to determine the total calcium in serum, plasma, or whole blood it is necessary to destroy the organic constituents, and then analyze the ash for calcium

## REAGENTS

- 1 Heparin (Hynson, Westcott and Dunning)
- 2 Four per cent Ammonium Oxalate Solution Dissolve 40 gm. of pure ammonium oxalate in water and dilute to one liter

3 Saturated Solution of Calcium Oxalate in 0.5 per cent  $\text{NH}_4\text{OH}$  Precipitate some calcium oxalate from a calcium chloride solution with an excess of ammonium oxalate. Then wash by decantation and centrifuging until the washings are free from excess oxalate and  $\text{NH}_4\text{Cl}$ . The filtered off precipitate is then transferred to the 0.5 per cent  $\text{NH}_4\text{OH}$  solution (prepared by diluting 2 cc of  $\text{NH}_4\text{OH}$ , sp gr 0.90 to 100 cc) and shaken for one hour in a shaker. It is next allowed to settle and the supernatant liquid filtered through a quantitative filter paper. The residual calcium oxalate may be used with a fresh lot of 0.5 per cent  $\text{NH}_4\text{OH}$  solution.

4 Approximately N H  $\text{SO}_4$ . Dilute 26 cc of concentrated H  $\text{SO}_4$  (sp gr 1.84) to one liter.

5 Standard N/100 Potassium Permanganate Solution. Dissolve 0.316 gm of pure potassium permanganate in redistilled water and dilute to one liter. After standing for several days standardize against sodium oxalate.

For the standardization proceed as follows. Dry sodium oxalate of the highest purity in an oven at 100 to 105° C for ten to twelve hours. Weigh off accurately, exactly 0.67 gm thereof and dissolve in redistilled water add 5 cc of concentrated H  $\text{SO}_4$  and dilute to one liter. This is a N/100 sodium oxalate solution. By means of a burette measure off 20 cc portions thereof into 100 cc Erlenmeyer flasks add 1 cc of concentrated H  $\text{SO}_4$  to each warm to about 70° C and titrate while hot with the  $\text{KMnO}_4$  solution. Determine the correction factor on the  $\text{KMnO}_4$  solution. It is necessary to restandardize the  $\text{KMnO}_4$  frequently \* but the Na  $\text{C}_2\text{O}_4$  solution with the H  $\text{SO}_4$  present is said to keep indefinitely. One cc of the N/100 solution is equivalent to 0.2 mg of calcium.

Procedure. To 2 cc of serum or heparinized plasma in a 15 cc centrifuge tube add 2 cc of distilled water. Mix well and then add 1 cc of the 4 per cent ammonium oxalate.

Again mix well and set aside for one hour. The centrifuge tube should have as fine a tip as possible. 3 to 4 mm in diameter at the tip is a satisfactory size. After standing for one hour the tube is covered with a rubber cap and centrifuged for ten minutes at high speed about 2300 revolutions per minute. The supernatant solution is removed either by decantation and finally inverting the tube for a moment upon filter paper or by siphoning the supernatant fluid out by applying suction to a pipette drawn out to a very fine point. The latter may be the better procedure in case the centrifuge tube has too large a diameter at the tip. Next the sides of the tube are washed down with 2 cc of the saturated  $\text{CaC}_2\text{O}_4$  solution in 0.5 per cent  $\text{NH}_4\text{OH}$  and the precipitate stirred up with a very fine glass rod. The rod is then rinsed off with 2 cc more of the 0.5  $\text{NH}_4\text{OH}$  solution. The centrifuging is repeated in the same way, and so also the decanting. The washing and decanting is repeated twice more making a total of three washings. After the last decanting add 2 cc of the H  $\text{SO}_4$ , break up the precipitate with a fine glass rod, and then heat the tube for two minutes in a bath of boiling water preferably in a

\* We have found however that N/100  $\text{KMnO}_4$  prepared from old stock N/10  $\text{KMnO}_4$  solution and kept at ordinary room temperature protected from light dust and outside air changes value much more slowly than freshly prepared N/100  $\text{KMnO}_4$  solution.

white porcelain dish so as to give a good white background to help in detecting the end point. While keeping the mixture hot (not over 70° C), titrate with the N/100  $\text{KMnO}_4$  solution by means of a microburette\* graduated to  $\frac{1}{100}$  c c and provided with a very fine point so as to deliver 100 drops or more per cubic centimeter. The first detectable pink color which remains for one minute after mixing is taken as the end point. Make a blank titration on 2 c c of  $\text{NH}_2\text{SO}_4$ . Deduct this from your known titration, and calculate the milligrams of Ca per 100 c c of plasma. This is calculated by the formula

$$\frac{(\text{Titration minus Blank}) \times \text{correction factor} \times 0.2 \times 100}{2}$$

or

$$(\text{Titration minus Blank}) \times \text{correction factor} \times 10 = \text{mg of Ca per 100 c c of plasma}$$

#### CONCLUSIONS

1 The most dependable results are obtained when the precipitated calcium oxalate is washed three times with a saturated solution of calcium oxalate in 0.5 per cent  $\text{NH}_4\text{OH}$ .

2 A microburette of the type mentioned is very convenient, and adds to the sensitiveness of the method.

#### REFERENCES

- 1 Kramer, Benjamin, and Tisdall, F. F. J Biol Chem 47 475, 1921
- 2 Tisdall, F. F. J Biol Chem 56 439, 1923
- 3 Clark, E. P., and Collip, J. B. J Biol Chem 63 461, 1925
- 4 Koch, F. C. J Lab & Clin Med 77 774, 1926

### AN ACCURACY CHECK FOR METABOLISM APPARATUS

By HARRY M. JONES, M.D., Ph.D., CHICAGO, ILL.

WITH the wide increase, during the past ten years, in the use of the basal metabolic rate in medical practice, there has grown greater instead of less confusion regarding the accuracy of the various methods and devices now used for determining the basal metabolic rate.

A standard recognized procedure for testing the accuracy of a given method or device, directly in terms of known physical chemical units and indirectly by comparing it with other devices whose accuracy has been similarly checked, could assist materially in clearing up this confusion and doubt regarding the accuracy of the various devices now available for making basal metabolic rate determinations.

The first very practical use for such a standard procedure would be, not so much for the purpose of checking the accuracy of the apparatus when received new from its manufacturer, but more especially for determining

\*A microburette devised by one of us (F. C. Koch) is a very convenient form of apparatus for this purpose. The reagent supply reservoir and connecting tube painted black insures a ready supply of reagent protected from light and dust. This burette is manufactured by Central Scientific Company, Chicago, Illinois.

whether or not the accuracy it possesses when new continues as time goes on. An apparatus may be accurate when first installed, but certain types of them rarely continue to remain so, for the reason that the causes of error are of such a nature that hardly a week goes by that one or the other of these sources of inaccuracy does not appear, their number and seriousness increasing as time goes on. In other words it is not so important to test the original accuracy as it is to see if this accuracy continues.

A second most important use for such a standard procedure if made simple enough for the average laboratory technician to understand and use, would be to serve as a stimulus to more painstaking technique in routine tests on the human subject. The too commonly careless or poorly trained technician needs a strict taskmaster. Any technician, whether well or poorly trained, knows that his metabolic rate determinations on the human patient must be accepted without question. There may be those who doubt the findings as stated in his report, but in the absence of other proof too often his report, right or wrong, stands.

But imagine that same technician put to the task of proving an already proved fact of chemistry, namely that a given quantity of alcohol requires its exact chemical equivalent of oxygen for its combustion. In other words, let him make a metabolism test on a device with a machine made metabolic rate, a rate known to be within at least one half of one per cent. The experiment challenges his best skill and by compelling his strictest attention to details develops in him, unawares, a practically flawless technique itself a most essential requirement for accuracy in the ordinary routine of metabolic rate determination on the human subject as well.

A third very practical use for such a standard procedure is in teaching the theory and practice of the subject of metabolism to medical students. Practically every medical school in this country today provides somewhere in its curriculum opportunity for the student to become acquainted by personal experience with some device or other used for making the test.

The importance of attention to technical details, however, cannot be sufficiently impressed upon the student for the reason that the human subject used for making the student demonstration test always has an unknown metabolic rate, varying from nothing to 100 per cent above the normal limits, depending upon his cooperation, emotional state and other factors introduced by the test subject. But the demonstration could be made with the accuracy of any other laboratory experiment, provided the student is required to make the test on a device of known metabolic rate, one not influenced by emotions, specific dynamic action of foods, lack of a sufficient period for rest and other known and unknown factors which in the human test subject, are causes of enormous fluctuations in the metabolic rate above and below the normal.

The student, having performed his laboratory experiment, using the alcohol cheek apparatus for his test subject, will then be required to review the chemical and physical factors involved in the calculation of a metabolic rate, to calculate the caloric value of the liter of oxygen to determine the respiratory quotient and its relation to the caloric value and to compare this heat output with that of the average normal human subject.



white porcelain dish so as to give a good white background to help in detecting the end point. While keeping the mixture hot (not over 70° C), titrate with the N/100  $\text{KMnO}_4$  solution by means of a microburette\* graduated to  $\frac{1}{100}$  c c and provided with a very fine point so as to deliver 100 drops or more per cubic centimeter. The first detectable pink color which remains for one minute after mixing is taken as the end point. Make a blank titration on 2 c c of  $\text{NH}_4\text{SO}_4$ . Deduct this from your known titration, and calculate the milligrams of Ca per 100 c c of plasma. This is calculated by the formula

$$\frac{(\text{Titration minus Blank}) \times \text{correction factor} \times 0.2 \times 100}{2}$$

or

$$(\text{Titration minus Blank}) \times \text{correction factor} \times 10 = \text{mg of Ca per 100 c c of plasma}$$

#### CONCLUSIONS

1 The most dependable results are obtained when the precipitated calcium oxalate is washed three times with a saturated solution of calcium oxalate in 0.5 per cent  $\text{NH}_4\text{OH}$ .

2 A microburette of the type mentioned is very convenient, and adds to the sensitiveness of the method.

#### REFERENCES

- 1 Kramer, Benjamin, and Tisdall, F. F. J. Biol. Chem. 47, 475, 1921
- 2 Tisdall, F. F. J. Biol. Chem. 56, 439, 1923
- 3 Clark, E. P., and Collip, J. B. J. Biol. Chem. 63, 461, 1925
- 4 Koch, F. C. J. Lab. & Clin. Med. 77, 774, 1926

### AN ACCURACY CHECK FOR METABOLISM APPARATUS

By HARRY M. JONES, M.D., PH.D., CHICAGO, ILL.

WITH the wide increase, during the past ten years, in the use of the basal metabolic rate in medical practice, there has grown greater instead of less confusion regarding the accuracy of the various methods and devices now used for determining the basal metabolic rate.

A standard recognized procedure for testing the accuracy of a given method or device, directly in terms of known physical-chemical units and indirectly by comparing it with other devices whose accuracy has been similarly checked, could assist materially in clearing up this confusion and doubt regarding the accuracy of the various devices now available for making basal metabolic rate determinations.

The first very practical use for such a standard procedure would be, not so much for the purpose of checking the accuracy of the apparatus when received new from its manufacturer, but more especially for determining

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whether or not the accuracy it possesses when new continues as time goes on. An apparatus may be accurate when first installed, but certain types of them rarely continue to remain so, for the reason that the causes of error are of such a nature that hardly a week goes by that one or the other of these sources of inaccuracy does not appear, their number and seriousness increasing as time goes on. In other words it is not so important to test the original accuracy as it is to see if this accuracy continues.

A second most important use for such a standard procedure if made simple enough for the average laboratory technician to understand and use would be to serve as a stimulus to more painstaking technique in routine tests on the human subject. The too commonly careless or poorly trained technician needs a strict taskmaster. Any technician, whether well or poorly trained, knows that his metabolic rate determinations on the human patient must be accepted without question. There may be those who doubt the findings as stated in his report, but in the absence of other proof too often his report, right or wrong stands.

But imagine that same technician put to the task of proving an already proved fact of chemistry, namely that a given quantity of alcohol requires its exact chemical equivalent of oxygen for its combustion. In other words, let him make a metabolism test on a device with a machine made metabolic rate a rate known to be within at least one half of one per cent. The experiment challenges his best skill and by compelling his strictest attention to details, develops in him unawares a practically flawless technique itself a most essential requirement for accuracy in the ordinary routine of metabolic rate determination on the human subject as well.

A third very practical use for such a standard procedure is in teaching the theory and practice of the subject of metabolism to medical students. Practically every medical school in this country today provides somewhere in its curriculum opportunity for the student to become acquainted by personal experience with some device or other used for making the test.

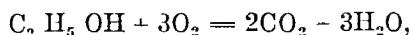
The importance of attention to technical details, however, cannot be sufficiently impressed upon the student for the reason that the human subject used for making the student demonstration test always has an unknown metabolic rate, varying from nothing to 100 per cent above the normal limits depending upon his cooperation, emotional state and other factors introduced by the test subject. But the demonstration could be made with the accuracy of any other laboratory experiment provided the student is required to make the test on a device of known metabolic rate, one not influenced by emotions, specific dynamic action of foods, lack of a sufficient period for rest and other known and unknown factors which in the human test subject are causes of enormous fluctuations in the metabolic rate above and below the normal.

The student, having performed his laboratory experiment, using the alcohol check apparatus for his test subject will then be required to review the chemical and physical factors involved in the calculation of a metabolic rate, to calculate the caloric value of the liter of oxygen, to determine the respiratory quotient and its relation to the caloric value and to compare this heat output with that of the average normal human subject.

This less complicated approach to the subject is made more readily understandable for the reason that in this experiment, only one fuel substance is involved, namely, alcohol, in the process of oxidation, instead of many substances in the oxidations going on in the human subject

The principle of the check method here described, familiar to researchers in metabolism work, is the so-called "alcohol check"

According to the chemical equation representing the burning of ethyl alcohol,



one molecular weight of alcohol (46 gm) requires for its combustion three molecular weights of oxygen, or 96 gm (i.e., 67.2 liters) of oxygen calculated as dry gas at 0° C and 760 mm Hg. Therefore, one standard liter of oxygen burns up 46 ÷ 67.2, i.e., 0.684 gm of absolute alcohol

A measured volume of alcohol is burned in a closed chamber to which is connected the metabolism apparatus about to be tested. The gases of combustion as they form are "breathed" by a pump into the metabolism apparatus. The oxygen, supporting the combustion being used to form CO<sub>2</sub> and H<sub>2</sub>O, and these, in turn, being absorbed by the soda lime, is thus gradually removed from the metabolism apparatus, and it is the entire purpose of the test to show that the amount of oxygen so removed is the exact chemical equivalent of the alcohol burned no more and no less

The value of the alcoholic check lies almost wholly in finding out the many reasons why the apparatus does *not* "check." Gas leaks, soda lime exhausted, or perhaps water soaked, spirometer improperly counterbalanced, or perhaps dented on one side, guide lines on tracing sheets printed out of line, too little water in spirometer, clogged breathing valves, ineffectual or too resistant to the breathing, and many other unsuspected sources of inaccuracy are brought to light to save nothing of the many sins of the operator himself

The alcoholic check device consists essentially of a gas-tight glass chamber provided with means for intake of oxygen and outflow of CO<sub>2</sub>, provided also with an alcohol burner so devised that it can be adjusted to different rates of alcohol flow. The quantity of heat generated by the flame, being approximately that given off by the average size adult, is dissipated by radiators, to eliminate errors in reading the oxygen volume which would occur if the gases in different portions of the circuit varied in their temperatures at the conclusion of the test, an error which could not be eliminated by calculation, i.e., correcting for this temperature change by taking the temperature of any one part of the circuit of gases. The rise in temperature, within the alcohol check apparatus itself, at the conclusion of any one test over its temperature at the beginning of that test is thus reduced by these radiators to a point too insignificant to take into account

The gases of combustion are alternately expired and inspired by means of a pump operated by an electric motor, the latter being adaptable, without change of any kind to direct or to 25 to 60 cycle type of alternating current

It is not necessary or even advisable, to use absolute alcohol. From about 85 to 90 per cent alcohol tends to remain at a fairly constant concentration with reasonable care in protecting it from contact with the air. The

percentage concentration of a given supply of alcohol may be determined by the specific gravity method accurate to about one tenth of 1 per cent.

Variations in temperature are the commonest causes of variations in the check determinations. For each  $1^{\circ}\text{C}$  change in temperature there will be a 0.1 per cent variation in the volume of alcohol, as measured by the ordinary pipette. Each  $1^{\circ}\text{C}$  change in temperature will cause a 0.4 per cent variation in the volume of oxygen.

With these variations due to temperature changes, taken into account and with the rate of ventilation (respiration) set at a constant rate other influences quickly become constants after the first few seconds from the beginning of the experiment.

The tube feeding the alcohol flame is of such caliber that one may easily read the volume of alcohol accurate to within one thousandth of a cubic centimeter.

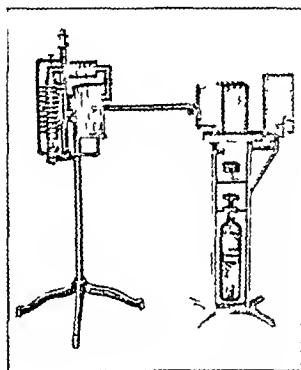


Fig. 1 — Alcohol check device connected to basal metabolism apparatus

The basal metabolism apparatus attached to the alcohol check apparatus (see Fig. 1) is so calibrated that one may read the volume of oxygen accurate to within two one thousandths of the standard liter of oxygen. The combined variations of both instruments will thus be not more than three parts out of one thousand or three tenths of 1 per cent.

It has been taught and is commonly believed, that the alcohol check on a metabolism apparatus cannot be closer than about 3 per cent. This teaching is incorrect. It is possible to measure off a quantity of liquid (alcohol) to match up with its chemical equivalent of a gas (oxygen) with all the precision and exactness of any chemical experiment, and this should certainly be, and is, less than 3 per cent of the theoretical.

The results of check determinations made with this apparatus at the University of Illinois College of Medicine on various types of basal metabolism apparatus will be the subject of a future report.

# A STUDY OF THE PRESSOR METHOD FOR THE STANDARDIZATION OF PITUITARY EXTRACT\*

BY EDWARD E SWANSON, INDIANAPOLIS, IND

## I REVIEW OF LITERATURE

THE need of accurate methods of standardization of pituitary extract for its uterine activity (oxytocic) and blood pressure raising activity (pressor) demands more technical study of the methods, especially is it so since there has been considerable discussion and controversy as to whether the physiologic properties of the posterior portion of the pituitary gland are due to more than one principle. Dudley<sup>1</sup> found that continuous extraction of the purified pituitary solution with butyl alcohol at reduced pressure yields a residue that contains all the uterine principle and a part of the pressor principle. Later Dudley<sup>2</sup> gave evidence for the presence of at least three different physiologically active principles in the posterior gland. Fuhner<sup>3</sup> reported the isolation of four crystalline principles. Abel and Kubota,<sup>4</sup> Abel and Nagayama<sup>5</sup> reported that the oxytocic-pressor actions of the posterior gland were due to one active specific substance or hormone. Abel and Rouiller<sup>6</sup> obtained results by a high purification of the infundibulum, which led them to believe that vasomotor oxytocic, and renal activities are only the physiologic actions of one and the same hormone. Abel, Rouiller and Geiling<sup>7</sup> and Abel,<sup>8</sup> by still greater purification concluded that the oxytocic-pressor, diuretic-antidiuretic, and respiratory activities were properties of one and the same substance. Smith and McClosky<sup>9, 10</sup> found that the oxytocic, pressor and renal actions had the same diffusion rate through a series of collodion membranes of graded permeability, and quantitatively determined the ratio of renal activity of their standard powder as compared with the fresh gland extract to be the same as the oxytocic-pressor ratio, that is to say, 1:7 weight for weight. These results led them to believe that the oxytocic-pressor-renal actions are the properties of one hormone. Hogben and Schlapp<sup>11</sup> found that the substance responsible for the consistent depressor action of the extract on the avian circulation is not the same principle which produces depression in the dog or cat, nor is it histamine. Hogben<sup>12</sup> later found that the substance present in the extract causing a powerful depressor action in the bird is a specific property of the fresh gland and appears to be associated like the pressor (mammalian) substance with the pars nervosa rather than the pars intermedia. Hogben and deBeer<sup>13</sup> found that the extracts of the pars intermedia have a higher oxytocic value than pressor value, as compared with the extracts of the pars nervosa. Schlapp<sup>14</sup> verified Dudley's work of extraction with butyl alcohol, and found that the quantity of pressor and melanophore substance or substances, exceeded that of the oxytocic substance. He therefore concluded that the oxytocic response and pressor-

\*From the Research Laboratories E.H. Lilly & Co. Indianapolis, Ind.  
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melanophore activities must be attributed to distinctly separate principles. However, there is no positive evidence that the pressor melanophore actions are due to distinct substances.

The above controversy in regard to the theories of unitarian and multiple activities of the posterior gland has recently been reported by Kamm, Aldrich, Grote, Rowe and Bugbee<sup>1</sup> in favor of the multiple theory, these authors have separated chemically the oxytocic and pressor hormones, by purification and fractionation with solvents and precipitants and have quantitatively shown their separation by the oxytocic and pressor methods of assay.

The physiologic properties of the principles of the posterior body are easily destroyed or decomposed by strong acids, alkalies, trypsin, and heat. Abel and Kubota,<sup>4</sup> Abel and Nagayama<sup>5</sup> heated extracts with HCl or boiled them in reflux condensers destroying completely the pressor principle but not all the oxytocic principle. Dudley and Dale and Schlapp later confirmed the same destructive action. Recently Stasial<sup>10</sup> by boiling or fractional sterilization at various hydrogen ion concentrations with HCl and acetic acid, found that the  $P_H$  controls the stability of the oxytocic property. This data would have been more conclusive if the pressor value had also been determined.

## II. THE PARTIAL SEPARATION OF THE OXYTOCIC AND PRESSOR PRINCIPLES IN THE MANUFACTURE OF PITUITARY EXTRACT

In the manufacture of pituitary extract which is the separation of the posterior portion from the anterior portion, the posterior portion is extracted with an acid water solution. This solution is purified by fractional precipitation, isoelectric precipitation etc. until a pure extract is obtained. The assays of the different fractions or precipitates show by the oxytocic and pressor methods of standardization a distinct difference in the pressor and oxytocic action. The final solution of pituitary extract has a balanced oxytocic pressor value by mixing the fractions or precipitates containing the various amounts of oxytocic and pressor activity as determined by the oxytocic and pressor methods of assay. During the assay of the different fractions a 1:2, 1:3 and even 1:5 pressor oxytocic ratio and vice versa oxytocic pressor ratio has been obtained in the regular purification process of manufacture of pituitary extract, thus verifying a part of the work of Kamm<sup>12</sup> and his associates.

## III. THE TOLERANCE FACTOR

Hogben, Schlapp, and McDonald<sup>17</sup> in their excellent report on the pressor method state, "The results of our experiments have been to show that after a lapse of time, varying with the size of the dose given full response can be recovered when complete immunity has been produced by repeated injections and it became evident, at an early stage in the investigation, that by administering the extract only at the end of the period adequate for complete recovery a constant response can be obtained over periods of many hours." This period of constant response they found to be one hour and the amount injected was approximately one half of the dose that will give a maximum response. As Dale<sup>18</sup> suggested the rate of excretion or destruction or establishment of immunity period, may depend upon the size of the dose given. If Hogben and

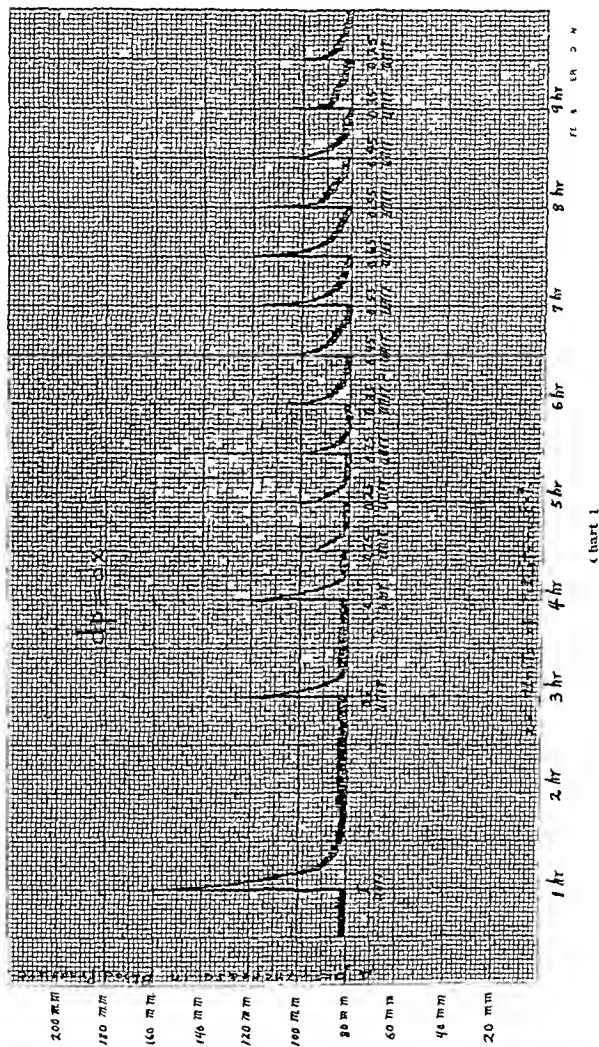
his coworkers had given injections at thirty minute intervals, the amount of pituitary extract injected would be less than is required for the hourly interval period. The increase in blood pressure or contractions would be less, thus at half hour intervals as well as at hourly intervals there would be a point of inflexion where the  $dp = dx$  is a maximum. To illustrate in Chart 1, if a cat requires two hours to establish immunity, or a period of constant response, with an injection of 1 unit, one hour for a 0.5 unit injection, thus 0.25 unit injection at thirty minute intervals should establish a period of immunity with a sufficient margin of response to allow larger or smaller injections or units to be given to obtain a quantitative assay equal in accuracy to the one hour period. This plan has been used in the following study of the spinal and anesthetic cats in the standardization of pituitary extract by the pressor methods.

#### IV THE INTERNATIONAL UNIT

The League of Nations (page 14 of a report by the Second International Conference on the Biologic Standardization of Remedies, Geneva, 1925) expressed the activity of a solution of pituitary extract in terms of international units each c.c. being equivalent to 10 international oxytocic units. The official solution of pituitary extract of the U.S.P. represents in each c.c. the equivalent of 5 mg. of a standard powder, which may be obtained from the U.S. Bureau of Chemistry. Each mg. of this standard powder contains 2 international oxytocic units. The U.S.P. solution of pituitary extract should contain 10 international oxytocic units, no requirement being made as to the pressor units. This standard powder was used in the study of the pressor method, 1 mg. being considered equal to 2 pressor units.

#### V THE PRESSOR METHOD OF STANDARDIZATION

*A. Spinal Cat Method*—Considering the above review and discussions of the literature, the definite proof of two principles in the posterior gland, the similarity in response to purification methods of these principles, and their possible injury by chemical reagents the need of accurate methods of standardization is indeed important. The oxytocic method has been extensively studied by Dale and Laidlow,<sup>19</sup> Dale and Burn,<sup>20</sup> Roth,<sup>21</sup> Pittenger,<sup>22</sup> Spaeth,<sup>23</sup> Eckler,<sup>24</sup> Nelson,<sup>25</sup> Hamilton and Rowe,<sup>26</sup> Hamilton,<sup>27</sup> Rowe,<sup>28</sup> and Swanson.<sup>29</sup> The pressor method has been reported by Hamilton and Rowe<sup>6</sup> and more recently by Hogben, Schlapp and McDonald.<sup>17</sup> These later workers describe a method on spinal cats, which is essentially that of the Dale and Laidlow<sup>19</sup> spinal cat method in studying the depressor and pressor properties of the pituitary gland. They have applied the spinal cat method as a quantitative method for determining the pressor value of pituitary extract. To briefly quote: "The cord is exposed under deep anesthesia by clipping off the neural arch of the axis vertebra the aperture in which is plugged plasticine after transection of the cord and destruction of brain. No injection made until two or three hours after the operation. The animal should be kept warm by wrapping, a rectal temperature of 35° C. to 37° C. maintained. If properly inflated and special importance is to be attached to the degree of insufflation a cat prepared in this way will maintain a blood pressure of 80 mg. for twenty-four hours. Each in-



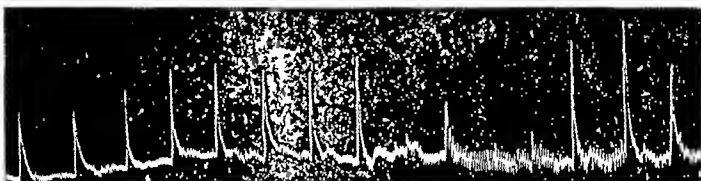
(chart 1)





Fig I represents one of many spinal cats with injections given every half hour. The first ten injections are alternating injections of Sample A (solution of pituitary extract containing 24 international oxytocic units and 24 pressor units), and Sample B (government standard powder No 232 each mg equivalent to 2 international oxytocic units and 2 pressor units and a prepared solution of this powder as directed by the U.S.P. so that each 1 cc contains 1 mg of this powder). The remaining contractions are gradual 10 per cent increase in the injections of Sample A. This tracing shows an accuracy of 10 per cent variation in the amount of pituitary extract.

Fig II represents a spinal cat with injections given every half hour. The injections are alternating doses of Sample A (24 oxytocic and pressor units) and Sample C (12 oxytocic and pressor units). The tracing shows that Sample C is one half as active as Sample A and Sample A contains the equivalent of 12 mg of Sample B (government powder) or 24 units (oxytocic and pressor).



0.1%	0.125%	0.15%	0.175%	0.16%	0.14%	0.12%	0.1%	0.08%	0.05%	0.025%	10	0.1%	0.1%	0.06
1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10
ST	ST	ST	ST	ST	ST	ST	ST	ST	W74X	W74X	W741Y	W741Y	ST	W74AA
11.00	11.30	12.00	12.30	1.00	1.30	2.00	2.30	3.00	3.30	4.00	4.30	5.00	5.30	6.00

MALE CAT - WEIGHT 2.4 KILOGRAMS AMYTAL 60 MILEGRAMS INTRAPERITONEALLY - VAGI INTACT

FIGURE III

*B The Anesthetic Cat Method*—For commercial standardization the anesthetized dogs or cats are more practical, in that it is comparatively simple to produce or prepare an animal with anesthesia. There are many anesthetics that may be used chloroform, paraldehyde, urethane etc., these substances produce a deep depressing anesthesia with low blood pressure, which gives small contractions or increase in blood pressure by the repeated injections of pituitary extract. The injections of pituitary extract must be small, to give consistent quantitative increases or contractions in the blood pressure. Too large doses of extract will develop tolerance with a gradual decrease in response or contractions and later a depressor action. More recently the barbital series as the sodium salt have been used as an anesthetic in pharmacologic study. These compounds do not give such a depression on the heart and blood pressure as compared to chloroform etc.

The following technique has been used with comparatively good results in the standardization of pituitary extract by the pressor method. The cat is weighed, injected intraperitoneally with 60 mg of amytal (isoamylethyl barbital acid) prepared in solution as the sodium salt as follows: 1 gm of

amytal is added to 8.85 cc  $\frac{1}{2}$  NaOH, heated to 80° C for fifteen minutes diluted to 10 cc with distilled water, and finally filtered. *This solution should be made fresh whenever needed.* The injection of 60 mg of amytal as the sodium salt per kg will produce anesthesia in thirty minutes. Some cats require more than 60 mg, but the average dose is approximately 60 mg per kg. Following the exposure of the carotid artery, femoral vein, and the adjustment of cannula and manometer, the reflexes should be observed. Page and Coryllos,<sup>30</sup> Swanson and Page,<sup>31</sup> and Edwards and Page<sup>32</sup> found that certain reflexes will indicate the degree of anesthesia. The sensory reflexes should be absent and only a slight corneal or conjunctival and knee jerk reflexes may be present. The blood pressure will maintain an approximate height of 100 mm to 120 mm. The injections of pituitary extract are given at half hour intervals,

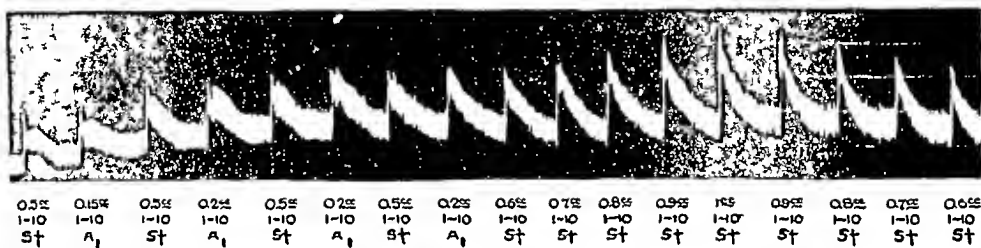


FIGURE IV

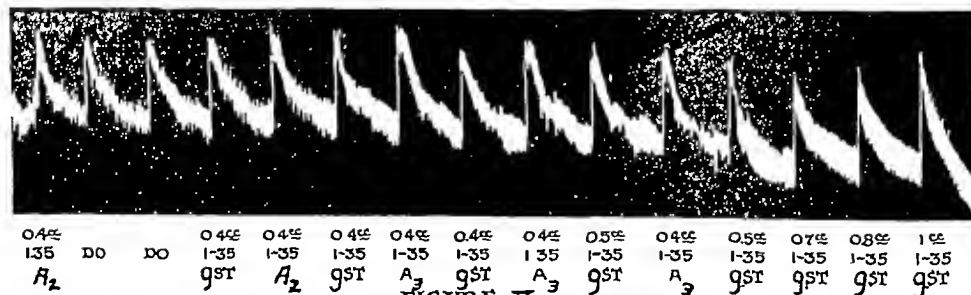


FIGURE V

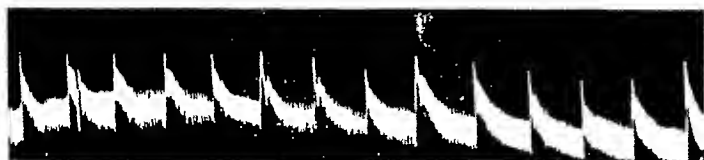
dilutions of 1 to 10 or 1 to 20 of a 10 international oxytocic unit solution preferred, the average dose being approximately 0.1 unit to 0.5 units depending upon the sensitiveness of the cat.

The following tracings represent a comparative assay of samples of pituitary extract prepared in our laboratories. Some of the samples represent a lot of pituitary extract in the process of manufacture, other samples represent dilutions of a known standardized solution, then dilutions of strength being unknown to myself.

Fig III is a very sensitive cat under anesthesia. The first eight injections are the standard solution (10 international oxytocic units per 1 cc) of 0.10, 0.125, 0.15, 0.175, 0.16, 0.14, 0.12, and 0.2 units respectively. These various amounts of units show a quantitative response in the increase of the blood pressure. W-74X, W-74XY, W-74AA are samples of certain fractions in the

process of a manufactured lot of pituitary extract. Fractions W 74X and W 74XY show far less response to increases of the blood pressure than W 74AA, W 74AA being approximately equal to the standard.

Fig IV represents the tracing of a cat (amytal anesthesia). This cat is not so sensitive as the cat in Fig. III requiring 0.5 units of the standard to produce consistent contractions. Sample A<sub>1</sub> is a solution of pituitary extract that is stronger than the standard. The first eight injections are alternating injections of the standard and Sample A. The amount of Sample A<sub>1</sub> increased until 0.2, (1 to 10) equals 0.5 pressor units of the standard. Therefore Sample A<sub>1</sub> contains about 25 pressor units. The remaining nine injections are increasing



MALE CAT WEIGHT 2.5 KILOGRAMS AMYTAL, INTRAPERITONEALLY VAGI CUT

FIGURE VI

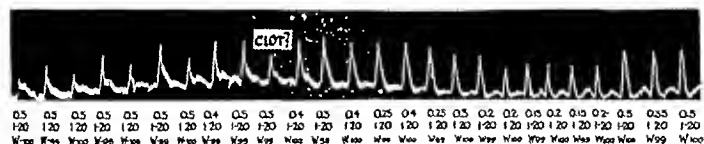


FIGURE VII

FEMALE CAT - WEIGHT 3 KILOGRAMS - VAGI CUT  
AMYTAL, INTRAPERITONEALLY

and decreasing amounts of the standard 0.6, 0.7, 0.8, 0.9, 1.0, 0.9, 0.8, 0.7 and 0.6 units respectively. These injections show a 10 per cent variation in response of contraction, and that it is possible to assay a pituitary extract solution by the pressor method within an accuracy of 10 per cent.

Fig V represents the tracing of a cat (amytal anesthesia) that is very sensitive. Samples A and A<sub>1</sub> are standard powders prepared by myself according to the method of the USP and the U. S. Bureau of Chemistry. These samples were compared with a Government standard powder No. 232 obtained from Dr. Munch of the Bureau of Chemistry. The tracing shows that Samples A and A<sub>1</sub> are equal to the Government Standard Powder or each mg. of powder is equivalent to 2 pressor units.

Fig VI represents the tracing of a cat (amytal anesthesia) vagi cut. W 96 and W 97 are samples of pituitary extract, their strength being unknown to

me Alternating injections of 1 cc (1 to 20) of W-96 and W-97 gave equal responses in the rise of blood pressure, therefore W-96 being equal to W-97. It was learned later that each sample was taken from the same lot of pituitary extract solution containing 10 international oxytocic units.

Fig VII represents the tracing of a cat (amytal anesthesia) vagi cut. W-99 and W-100 are samples of pituitary extract, their strength being unknown to me. Twenty-six alternating injections were given to this cat. Sample W-100 assayed 60 per cent to 70 per cent of W-99. The dilutions were made so that W-100 would be 66 $\frac{2}{3}$  per cent as active as W-99 which was a regular manufactured lot of pituitary extract containing 10 international oxytocic units.

Table I represents the results of the assayed samples, their strength in units both pressor and oxytocic values as determined by the pressor and oxytocic methods.

TABLE I

PREPARATION NO	MADE BY	PRESSOR UNITS	INTERNATIONAL OXYTIC UNITS	KNOWN PRESSOR OXYTIC UNITS
A Mfg lot	Walden	24	24	24
A <sub>1</sub> Mfg lot	Walden	25	25	--
A <sub>2</sub> St Powder	Swanson	1 mg = 2 units	1 mg = 2 units	--
A <sub>3</sub> St Powder	Swanson	1 mg = 2 units	1 mg = 2 units	--
B 232 Powder	Gort	1 mg = 2 units	1 mg = 2 units	1 mg = 2 units
C Mfg lot	Walden	12	12	--
W 97 Mfg lot	Walden	12	12	12
W 96 Mfg lot	Walden	12	12	12
W 100 Mfg lot	Walden	7.8	7.8	8
W 99 Mfg lot	Walden	12	12	12

## DISCUSSION

A discussion and study of the spinal and anesthetic methods of the standardization of the pressor principle of pituitary extract leads one to believe that either method is practical provided that injections are given at one-half hour intervals. The spinal cat is an excellent method, which eliminates the anesthetic factor, maintains a constant blood pressure level, and is very sensitive to injections of pituitary extract with distinct measurable contractions. The anesthetic cat is more practical in that the technique is more simple, and the anesthetic factor can be so regulated as to maintain an experiment that is as accurate as the spinal cat and will give an accuracy of 10 per cent variation.

Two samples of standard desiccated powder A<sub>2</sub> and A<sub>3</sub> prepared according to the U.S.P. assayed equal to a standard powder No. 232 received from the Bureau of Chemistry, each mg containing 2 pressor units by the pressor method of assay.

A few samples of pituitary extract, their strength being unknown to the operator, were tested by the pressor method, and an approximately accurate assay was obtained that agreed with the dilutions and strengths known to the individual who made the preparations.

The technique of producing a spinal or anesthetic cat requires care in preventing clots in the cannula which should be thoroughly cleaned, boiled in acid or cleaning solution inserted into the artery in such a way as to prevent any dirt or hairs coming in contact with the inner surface of the cannula.

## CONCLUSIONS

1 The partial separation of the oxytocic and pressor principles has been verified in the regular manufacture of pituitary extract

2 Spinal and anesthetic cats are both accurate methods of determining the pressor principle in units the anesthetic method being more practical in that the technique is simple

3 Several samples of pituitary extract were assayed by the spinal and anesthetic methods. The tests show considerable accuracy by these methods

## REFERENCES

- 1 Dudley J Pharmacol 14 295 1919
- 2 Dudley J Pharmacol 21 10 1923
- 3 Fahnor Ztschr f d ges exper Med 1 59 1913
- 4 Abel and Kubota J Pharmacol 13 41 1919
- 5 Abel and Nagayama Ibid 15 347 1920
- 6 Abel and Rouiller Ibid 20 65 1922
- 7 Abel Rouiller, and Geiling Ibid 22 259 1923 22 303 1923
- 8 Abel J Ind Eng Chem 16 1031 1924
- 9 Smith and McCloskey Hygiene Laboratory Bulletin 139 April 1924
- 10 Smith and McCloskey J Pharmacol 24 91 1924
- 11 Hogben, and Schlapp Quart J Exper Physiol 14 220 1924
- 12 Hogben Ibid 15 155 1925
- 13 Hogben and deBeer Ibid 15 163 1925
- 14 Schlapp Ibid 15 326 1925
- 15 Kamm Aldrich Grote Rowe and Bugbee J Am Chem Soc 50 573 1928
- 16 Stasiak J Pharmacol 28 1 1926
- 17 Hogben Schlapp and McDonall Quart J Exper Physiol 14 315 1924
- 18 Dale Biochem J 4 427 1909
- 19 Dale, and Landlow J Pharmacol 4 75 1912
- 20 Dale and Burn Reports from the Dept of Bio Standards of Medical Res Council 1912
- 21 Roth J Pharmacol 5 559 1914
- 22 Pittenger J Am Pharm A 7 512 1918
- 23 Spaeth J Pharmacol 11 209 1918
- 24 Eckler J LAB & CLIN MED 2 819 1917
- 25 Nelson J LAB & CLIN MED 8 218 1923
- 26 Hamilton & Rowe J LAB & CLIN MED 2 120 1916
- 27 Hamilton J Am Pharm A 1 1117 1912
- 28 Rowe J Pharmacol 9 107 1916
- 29 Swanson J LAB & CLIN MED 9 334 1924
- 30 Page Corliss J Pharmacol 30 513 1926
- 31 Swanson Page Ibid 31 1 1927
- 32 Edwards Page Am J Physiol 76 207 1926
- 33 Edwards Page Ibid 78 235 1926

# A SIMPLE PRECIPITATION TEST FOR SYPHILIS WITH SMALL QUANTITIES OF DEFIBRINATED FINGER BLOOD\*

## PRELIMINARY REPORT

By B S KLINE, M.D., CLEVELAND OHIO

THE simple precipitation test for syphilis with a few drops of finger blood, described in this article, is as specific and as sensitive as the microscopic slide precipitation tests and the Wassermann test with the same antigen. Because it requires but a small quantity of comparatively easily obtainable blood, the finger blood precipitation test is particularly useful in the diagnosis of syphilis in infants and children. After its reliability is established by a sufficient number of comparative tests finger blood may be used routinely in the diagnosis of syphilis.

Finger blood in small quantities, as is well known, is generally employed in hemoglobin determinations, red blood cell and white blood cell counts, and in certain agglutination tests.

A rapid and simple hemagglutination test with small quantities of whole blood obtained from the finger, for use in testing donors for transfusion, was reported by Rous and Turner<sup>1</sup> in 1915. For the test a small quantity of blood is drawn from the finger of the patient into a Wright pipette containing 10 per cent sodium citrate. Blood is similarly obtained from the prospective donor. Two mixtures of these bloods are made: (a) 9 parts citrated blood of the patient to one part of citrated blood of the donor, (b) one part citrated blood of the patient to one part of citrated blood of the donor. The mixtures are made in Wright pipettes (from pipette to slide on Widal plate and back a number of times), and read two to fifteen minutes after preparation.

Whole blood obtained from the finger has been extensively employed in the Widal reaction since 1896 when Wyatt Johnson<sup>2</sup> reported its reliability for the detection of typhoid agglutinins. In this method, single drops of blood are collected on glass, cardboard, or paper and subsequently (as late as forty-four days) diluted with sterile water. One drop of the whole blood solution and one drop of a suspension of typhoid bacilli are mixed on a cover slip and this is inverted and sealed over a chamber. Readings are made fifteen minutes to one hour after preparation.

An ideally simple yet thoroughly reliable finger blood test for agglutinins was introduced by Bass and Watkins<sup>3</sup> in 1910. In this test approximately one-fourth of a drop of blood is spread on a microscopic slide (with the end of another slide). Immediately after making the spread, or sometime after the blood has dried, one drop of water is allowed to fall on the slide and is spread over the surface by a toothpick. As soon as the blood has dissolved (less than one minute), one drop of a suspension of typhoid bacilli is added, the mixture is agitated by tilting the slide from side to side for two minutes, and the reac-

\*From the Mount Sinai Hospital

tion then noted. The use of an open microscopic slide and the employment of agitation to accelerate the reaction are advantages of simplicity and rapidity not possessed by the other Widal tests.

No reference to a test for syphilis with small quantities of defibrinated blood has been found in the literature.

#### MATERIALS FOR THE FINCH BLOOD SPEECHIFICATION TEST FOR SYPHILIS (SEE FIGS. 1 AND 2)

1 Micro Culture Slides (Will Corporation No 11802)  $3 \times 1$  inch with wells 16 mm  $\times$  3 mm for collecting the finger blood.

2 Toothpicks with flat ends for defibrinating the blood in the chambers.

3 Humidor (box with tightly fitting lid containing wet blotter) to keep the defibrinated blood until tested.

4 Microscopic slides  $3 \times 2$  inches each with two double rings made as follows. A  $3 \times 2$  inch microscopic slide is covered on both sides with Bon Ami paste. After the paste has dried (in a few minutes) it is rubbed off with a clean soft muslin cloth leaving a clean smooth surface. Upon the clean slide toward one end a non harness ring  $1\frac{1}{4}$  inches inside diameter  $\frac{3}{16}$  of an inch thick (obtainable at hardware stores) is placed. In its center a No 13 cork or a No 6 rubber stopper is placed. The well between them is filled with hot wax (two parts of ordinary vaseline and one part of parowax) from a 10 c.c. glass syringe. After the mixture cools a few minutes the cork and ring are removed and the process repeated at the other end of the slide. Rings made with the mixture given above adhere well to the slide whereas rings of parowax alone occasionally separate from the slide at low room temperatures and allow the fluid to escape.

A better method of making the outer rings is as follows. Upon the clean slide a steel mold  $3 \times 2 \times \frac{1}{8}$  inches with two central wells  $3\frac{1}{4}$  cm. in diameter is placed. A metal disc  $1\frac{1}{4}$  inches in diameter and  $\frac{1}{16}$  of an inch thick is then placed in the center of each well. The space between them is filled with hot wax from a 10 c.c. glass syringe. After the mixture cools a few minutes the mold is removed by inserting a thin blade between it and the slide. After the outer rings are made ( $3\frac{1}{4}$  cm. outside diameter and 2.9 cm. inside diameter) the inner rings (15 mm. inside diameter) are made with hot parowax by means of a thread wound wire loop 16 mm. inside diameter devised by Green.\*

5 Slide holders (for three  $3 \times 2$  inch slides). The slide holder is a wooden lid of a slide box ( $3\frac{3}{4} \times 6\frac{3}{4}$  inches) containing an easily fitting thin wooden shelf having a small handle at each end made with a wire paper clamp (about one third of the clamp is fastened under the shelf with adhesive tape and the remainder after being forced through the shelf is bent to serve as a handle).

6 Holder for two slide holders consists of a wooden lid ( $13\frac{5}{8} \times 3\frac{3}{8}$  inches inside measurements  $14\frac{1}{2} \times 4$  inches outside measurements) making it possible to use six slides at one time.

7 Ordinary 1 c.c. pipettes (graduated in hundredths) or 0.02 c.c. pipettes (graduated in thousandths) for pipetting the defibrinated blood.

8 Wright pipettes for delivering drops 0.015 to 0.016 c.c. of 7 per cent sodium chloride solution and others to deliver drops 0.017 to 0.018 c.c. of



antigen emulsion These pipettes may be made from ordinary glass tubing (10 mm outside diameter) or from portions of 10 cc pipettes by drawing out one end into a fine tube of the proper diameter A rubber bulb is used to control the pipetting If a calibrated pipette is used the quantity of the drop can be determined readily If ordinary tubing is used, the following method of determining the proper sized drop is employed 0.5 cc of 7 per cent sodium chloride solution or 0.5 cc of antigen emulsion is pipetted into a small container The fluid is quickly drawn up into the pipette to be tested The pipette is held vertically and slight pressure is exerted on the bulb to force drops out slowly A satisfactory pipette for the antigen emulsions will deliver 27 to 29 drops per 0.5 cc and a satisfactory pipette for the 7 per cent sodium chloride solution will deliver 31 to 33 drops per 0.5 cc

9 Antigen emulsions The antigen employed is one described previously Briefly the details of its preparation are as follows

Two hundred grams of dried heart powder (Difco) is placed in a two liter Erlenmeyer flask One liter of absolute ethyl alcohol (99 plus per cent) is added

After the flask is stoppered with a cork covered with tin foil it is shaken vigorously by hand for from twenty to thirty minutes (This short extraction removes almost all of the desired antigenic substance in the powder) A shaking machine may be employed for the extraction After a few hours in the machine practically all the antigenic substance is extracted

The extract is filtered into a liter cylinder through good grade filter paper of medium texture (Schleicher and Schuell No. 597, 38 cm)

During filtration, the mixture is stirred with a wooden tongue depressor and toward the end, pressed with a cork until the powder is quite dry

The extract (about 775 cc) is placed in the refrigerator at 8° to 10° C for from fifteen to forty-eight hours

During this time, a fairly heavy white precipitate settles out This is filtered off and the filtrate, in a large evaporating dish, is concentrated on a water-bath or by an air heater (bathroom heater) at 15° to 50° C determined by a thermometer bulb within the extract As the alcoholic extract evaporates down, there is an irregular festoon at the periphery until the desirable concentration (about  $\frac{1}{20}$  of the original volume) is reached At this time the festoon disappears and the margin of the concentrated extract is sharp The extract is now ready to be poured into acetone

The extract is poured into 500 cc of acetone c.p. at 50° C in a large evaporating dish This is then placed in an incubator at 37° C until the temperature of the fluid reaches this level After this it is allowed to stand at room temperature for several minutes when the acetone is decanted leaving a soft yellow brown wax-like substance adherent to the dish The lipid is then worked together and placed in a glass stoppered bottle with 80 cc of absolute ethyl alcohol (99 plus per cent) at about 50° C

After a few minutes' shaking, the bottle is placed in an oven (at 50° to 56° C) for thirty minutes to dissolve as much of the lipid as possible

On removing the bottle from the oven it is shaken for a few minutes and then placed in the refrigerator at 8° to 10° C for forty-five minutes

The solution is then filtered and the filtrate is evaporated down at 45° to 50° C (water bath or oil heater) resulting in a soft brown wax like substance (antigen lipid). The antigen lipid is weighed and to each gram, in a glass stoppered bottle 10 c.c. of absolute ethyl alcohol (99 plus per cent) is added. After the bottle is shaken for a few minutes it is placed at 50° to 56° C for thirty minutes, and then shaken a few minutes. (The lipid although obtained from a clear alcoholic solution is again incompletely soluble in alcohol.)

The slightly turbid solution is placed at 8° to 10° C for an hour, and then filtered. The resultant clear filtrate is the antigen and contains about 8.75 per cent of the alcohol treated acetone insoluble lipid. (This lipid, if repeatedly treated with alcohol or precipitated with acetone, is still incompletely soluble in alcohol.)

The antigen is kept at room temperature.

With the antigen described above emulsions may be prepared having the following advantages over Kahn antigen dilutions.

- | Antigen Emulsions                                                                                     | Kahn Antigen Dilutions                                                            |
|-------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| (a) Relatively stable satisfactory for at least three days                                            | (a) Unstable satisfactory for an hour or an hour and a half at most, usually less |
| (b) Satisfactory at low room temperatures as well as at ordinary ones                                 | (b) Majority unsatisfactory at low room temperatures                              |
| (c) The very sensitive emulsion is more sensitive than the Kahn antigen dilution and no less specific |                                                                                   |
| (d) The emulsions are more uniform in quality and quantity than the Kahn antigen dilutions            |                                                                                   |

The formula for the finger blood precipitation test antigen emulsion is as follows:

- 0.85 c.c. Distilled Water
- 0.5 c.c. 1 per cent Cholesterol (C. P. Pfanstiehl) solution in absolute (99 plus per cent) ethyl alcohol
- 0.1 c.c. Antigen
- 2.95 c.c. 0.85 per cent sodium chloride (Merck c.p. or reagent) solution

The emulsion is prepared in the following manner:

Into a one ounce bottle 0.85 c.c. distilled water is pipetted. The bottle is held at an angle and the 0.5 c.c. of 1 per cent cholesterol solution is allowed to run along the side of the bottle.

The bottle is gently rotated from the neck for from fifteen to twenty seconds.

The bottle is held at an angle again and 0.1 c.c. of antigen is pipetted against the side from a 0.2 c.c. pipette (graduated in thousandths).

The bottle is promptly stoppered with a cork and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for one minute.

Lastly, the 2.95 cc. of 0.85 per cent sodium chloride solution is allowed to run in quite rapidly. The bottle is stoppered again and shaken as previously for one minute.

The very sensitive antigen emulsion for the finger blood precipitation test consists of the emulsion just described treated in the following manner. About 1 cc. of sensitive antigen emulsion is placed in a rack in a large pan of water bath with the water at least two inches above the level of the emulsion. The water is maintained at 50° C. After twenty minutes at this temperature,

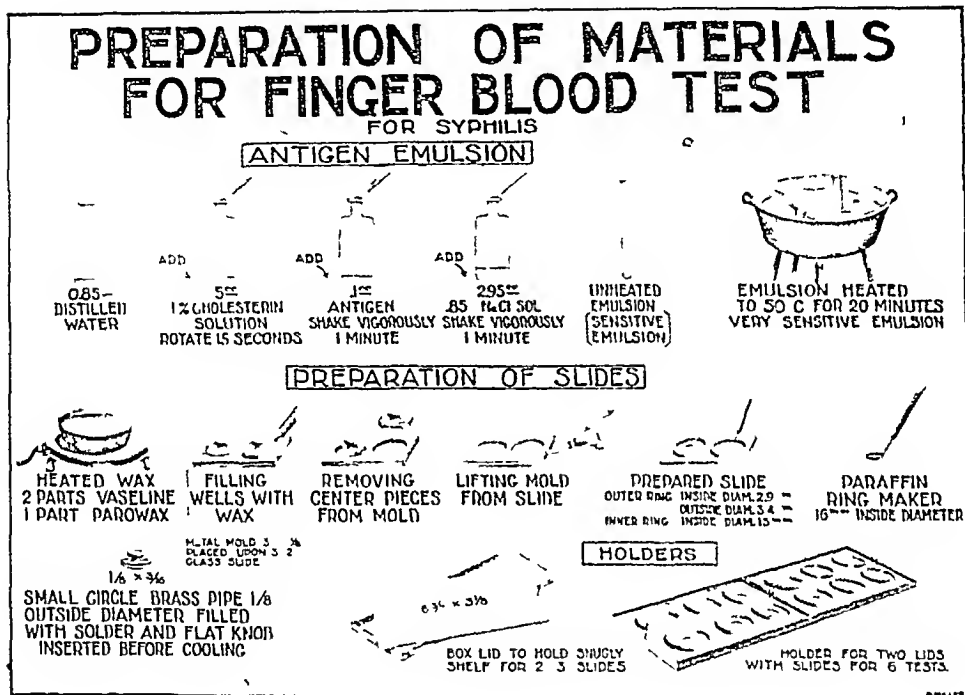


Fig. 1

the emulsion is removed and kept at room temperature until used (one-half hour to two hours after the original preparation of the emulsion).

The 1 per cent cholesterolin solution for the antigen emulsion is prepared as follows:

To 1 gram of cholesterolin (e. p. Pfanstiehl) 100 cc. of absolute ethyl alcohol (99 plus per cent) is added and the bottle placed in an incubator at 50° C. to 56° C. The bottle is shaken at fifteen-minute intervals to promote solution which is complete in about forty-five minutes. This stock solution is kept in the incubator at 37° C. and is perfectly satisfactory for the preparation of the emulsion for as long as two months.

**10 Defibrinated finger blood.** The patient's finger tip is cleaned with alcohol and one or two puncture wounds made with a sharp needle. With the patient's hand in a dependent position, the bleeding finger is squeezed with

both hands and the blood allowed to fall into the chamber of a micro culture slide resting upon a chin or other object. The blood in the chamber is then stirred with the flat end of a toothpick until defibrinated (several minutes). The slide is next labelled with a chin marking pencil placed in the humidor and kept (in the ice chamber at about  $10^{\circ}\text{C}$  preferably) until the blood is to be tested (from a few minutes to twenty four hours after collection).

#### FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS (SEE FIG. 2)

1 Pipette 0.04 cc of defibrinated finger blood obtained as described above into each inner ring of the  $3 \times 2$  inch slide.

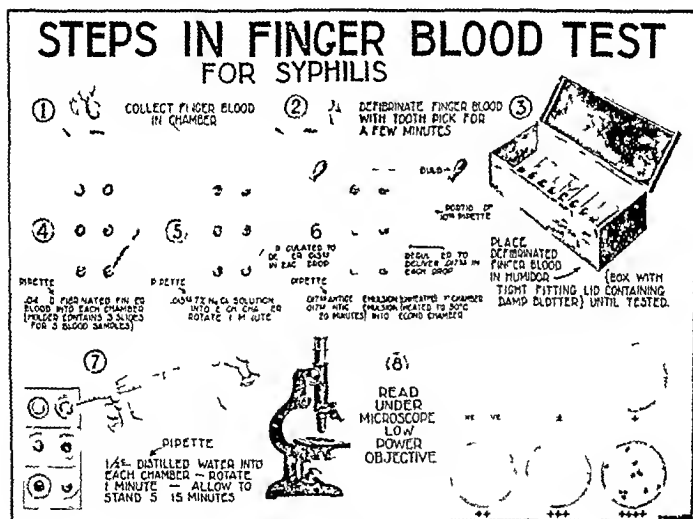


FIG. 2

2 Allow one drop (0.015 to 0.016 cc) of 7 per cent sodium chloride solution to fall (from a pipette described above) into each inner ring containing blood, and rotate the slide quite vigorously for one minute.

3 Allow one drop (0.017 to 0.018 cc) of sensitive antigen emulsion to fall (from a pipette described above) into the first blood salt mixture. In the same manner allow a similar quantity of very sensitive antigen emulsion to fall into the second blood salt mixture. Rotate the slide with moderate vigor for four minutes. (With proper sized rings and proper rotation, spilling from the inner chamber with consequent spoiling of the test will not occur.)

4 Allow 1.5 cc of distilled water to fall (from a 1.5 cc pipette) into each mixture (the mixtures spill over to the outer rings) and rotate the slide gently.

for one minute to completely lake the blood. Allow the slide to stand from five to fifteen minutes (ten minutes preferably) before reading.

5 Readings are made through the microscope at a magnification of about one hundred times (low power objective 16 mm eyepiece 12 X or 12½ X. A 10 X eyepiece is not as satisfactory). The light should be somewhat brighter than for reading the microscopic slide precipitation test with serum. The contents of the chambers are examined in different portions and at different levels, and read negative if clumps are absent and + to +++ depending upon the size of the clumps and upon the degree of the clumping (see Fig. 2). Leucocytes and any debris which may be present can be readily differentiated from the compact retractile yellow brown clumps of a positive reaction. If large clumps are present they may all be at the bottom of the fluid. Ordinarily, however, the clumps are quite evenly distributed in all levels. The negative tests contain granules but no clumps. To differentiate these granules from the small clumps of a + test, various portions are carefully examined at different levels by turning the large micrometer screw of the microscope very slowly.

Six blood specimens may be tested with the sensitive emulsion and with the very sensitive emulsion at one time by using a holder (see Fig. 1) containing two lids each of which carries three slides on a shell.

At the time blood was obtained from a finger for the test just described a larger quantity was drawn from an arm vein to furnish serum for the microscopic slide precipitation tests and for the Wassermann test. Fifty and four-tenths per cent of the finger bloods examined were obtained from hectic patients under treatment. Since the majority of these bloods gave + to +++ results, an excellent opportunity was afforded for a comparison of the sensitivity of the different tests. No false positive results occurred in any of the tests. The comparative results of the four tests with the same antigen are given in Tables I and II.

TABLE I

COMPARISON OF SENSITIVE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS WITH SENSITIVE SERUM TESTS

	FINGER BLOOD TEST AND SLIDE PRECIPITA- TION TEST (HEATED SERUM) <sup>a</sup> TESTS	PER CENT	FINGER BLOOD TEST AND SLIDE PRECIPITA- TION TEST (RAW SERUM)* TESTS	PER CENT	FINGER BLOOD TEST AND WASSERMANN TEST (CLEAN METHOD) (HEATED SERUM) TESTS	PER CENT
Agreement	319	82.64	127	88.81	215	76.24
Relative agreement	64	16.58	16	11.19	17	16.67
Agreement or relative agreement	383	99.22	143	100.00	262	92.91
Disagreement	3	0.78	0	0	17	6.03
Anticomplementary					3	1.06
Total	386		143		282	

17 Disagreements with Wassermann Test 12 False negative Wassermann Tests 5 1 also negative Finger Blood Precipitation Tests

3 Disagreements with Slide Precipitation Test (Heated Serum) 3 False negative Finger Blood Precipitation Tests

Evaluation according to the method of Kahn

Positive Reaction ++++ + + + and + +

Doubtful Reaction + and ±

Agreement positive negative or doubtful by both methods

Relative agreement positive or negative by one method and doubtful by the other

Disagreement positive by one method and negative by the other and vice versa

\*Slide precipitation test with raw serum to be described

Tables I and II show that the finger blood precipitation test for syphilis is as specific and as sensitive as the microscopic slide precipitation tests and the Wassermann test of serum obtained by venipuncture.

TABLE II

COMPARISON OF VEIL SENSITIVE FINCH BLOOD RECHITATION TEST FOR SYPHILIS WITH  
VEIL SENSITIVE SERM TESTS

	FINGER BLOOD TEST AND SLIDE FICIPITA TION TEST (HEATED SERUM) TESTS	PLP CENT	FINGER BLOOD TEST AND SLIDE FICIPITA TION TEST (FAW SERUM) TESTS	PER CENT	FINGER BLOOD TEST AND WASSELMANN TEST (LEVELAND METHOD) (HEATED SERUM) TESTS	1EP CENT
Agreement	5	8.64	6	8.54	17	76.24
Relative agreement	15	16.56	15	16.46	17	16.85
Agreement or relative agreement	110	100.00	79	100.00	94	93.07
Disagreement	0	0	0	0	6	5.94
Anticomplementary					1	0.99
Total	110		79		101	

6 Disagreements with Wassermann Test 4 False negative Finger Blood Precipitation Tests - False negative Wassermann Tests

## DISCUSSION OF THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

The principles underlying the Finger Blood Precipitation Test for syphilis are in part similar to those described by Kahn<sup>7</sup> for precipitation tests on heated serum. The following additional principles underlie the test with unheated blood: (1) Determination of the optimal quantity of electrolytes to secure satisfactory sensitivity. (2) Use of heat to increase the sensitivity of the antigen emulsion.

TABLE III

### INFLUENCE OF ELECTROLYTES ON THE PRECIPITATION REACTION

[illegible]

Fresh unheated positive defibrinated blood when tested as is heated serum gives strikingly less sensitive results. A simple method of securing satisfactory sensitivity with unheated blood is the addition of an optimal quantity of electrolytes. (See Table III.) Tests of defibrinated blood with various quantities of salt solution and antigen emulsions containing various quantities of cholesterol, showed that results equal to those of the sensitive precipitation test with heated serum followed the use of 0.015 to 0.016 c.c. of 7 per cent sodium chloride solution and 0.017 to 0.018 c.c. of antigen emulsion containing 0.5 c.c. of 1 per cent cholesterol solution in 4.4 c.c. of emulsion.

In the tests with positive unheated defibrinated blood, it was found that the antigen emulsion increases definitely in sensitivity for one-half hour after its preparation, that for the period one-half hour to two hours after its preparation there is no appreciable increase in sensitivity, that after two hours there

TABLE IV  
INFLUENCE OF HEAT ON THE SENSITIVITY OF THE ANTIGEN EMULSION

DEFIBRINATED BLOOD NO.	NOT HEATED	ANTIGEN EMULSION HEATED 20 MINUTES TO			
		42° C.	45° C.	47° C.	50° C.
1		++	+++		
2		±	+		
3		++++	++++		
4		++++	++++		
5		-	-		
6		-	-		
7		-	-		
8		-	-		
9		-	-		
10		-	-		
11	+		++, +++	+++	
12	±, +		++	+++	
13	±, +		+	+, ++	
14	++		+++	+++, +++++	
15	++		+++, +++++	+++, +++++	
16	-		-	-	
17	-		-	-	
18	-		-	-	
19	-		-	-	
20	-		-	-	
21	-		-	-	
22	-			±, +	+
23	-			±	±, +
24				±	±
25	-			±	±
26	±			±, +	+
27	±			+, ++	++
28	±			+, ++	++
29	±, +			+, ++, +++	+++
30	+			+, ++, +++	+++
31	++++			++++	++++
32	-			-	-
33	-			-	-
34	-			-	-
35	-			-	-
36	-			-	-
37	-			-	-
38	-			-	-
39	-			-	-
40	-			-	-
41	-			-	-

is again a detectable increase and that four to six hours after it is made, the emulsion is very sensitive and gives results with positive bloods about equal to those in the very sensitive serum slide tests and in the very sensitive Wassermann test with the same antigen. After reaching its maximum sensitivity in four to six hours the emulsion retains its antigenic power undiminished for at least twenty-four hours. In negative sera no clumps are formed at any time during this period.

The necessity for waiting four hours or longer for the emulsion to become very sensitive is obviated by heating the emulsion to 50° C for twenty minutes. The heated emulsion shows much less change on standing than the original sensitive emulsion and may safely be used for at least one and one-half hours. The influence of heat on the sensitivity of the emulsion is shown in Table IV. The quantity of blood (0.04 cc) used in the test is sufficient for accuracy and an amount that may be taken (with four times as much distilled water) within a reasonably small space.

The inner small chamber on the slide satisfies the necessity for mixing concentrated ingredients and the outer larger one for subsequently taking the red cells to make possible the reading of the results.

#### CONCLUSIONS

1 The simple precipitation test for syphilis with a few drops of defibrinated finger blood described above is as specific and as sensitive as the microscopic slide precipitation tests and the Wassermann test with the same antigen.

2 Because it requires but a small quantity of comparatively easily obtainable blood the finger blood precipitation test is particularly useful in the diagnosis of syphilis in infants and children.

3 After its reliability is established by a sufficient number of comparative tests finger blood may be used routinely in the diagnosis of syphilis.

The development of the finger blood precipitation test was made possible through the cooperation of the Staff of the Out Patient Department of the hospital. Most of the patients tested were referred through the courtesy of Dr S. Littman from the Department of Dermatology and Syphilology. Dr B. Levine of this department aided greatly in securing the specimens studied and in evaluating the clinical histories.

#### REFERENCES

- 1 Rous P. and Turner J. R. A Rapid and Simple Method of Testing Donors for Transfusions. *J. A. M. A.* 64: 1980 June 12 1912.
- 2 Johnson W. On the Application of the Serum Diagnosis of Typhoid Fever to the Requirements of Public Health Laboratories. *New York M. J.* 64: 573 October 1896.
- 3 Bass C. C. and Watkins J. A. A Quick Macroscopic Typhoid Agglutination Test. *Arch. Int. Med.* 6: 17 1910.
- 4 Green G. *Am. J. Pub. Health.* 15: 651 1925.
- 5 Kline B. S. An Antigen for Use in Serum Tests for Syphilis. *J. Lab. & Clin. Med.* 13: 588 March 1928.
- 6 Kline B. S. Young A. M. A Microscopic Slide Precipitation Test for Syphilis. *J. Lab. & Clin. Med.* 12: 477 February 1927.
- 7 Kahn R. L. Serum Diagnosis of Syphilis by Precipitation. Williams & Wilkins Company 1925.



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KIDUFFE M.D. ABSTRACT EDITOR

## LABORATORY TECHNIC

**PHENOBARBITAL POISONING** Toxicologic Analysis, Wright, H. N. Arch. Int. Med. 43 No. 1, 85, 1929

The following method of analysis is proposed

Hash tissue and extract from three to five times with ether

Filter

Residue, reserve for further extraction if necessary

Filtrate, concentrate  
Contains phenobarbital fats, soaps and some split proteins. Extract with dilute alkali

Ether layer  
Contains fats

Water layer  
Contains phenobarbital plus soluble soaps and split proteins. Make acid with acetic acid and extract with ether

Ether layer  
Contains fairly pure phenobarbital. Repeat extraction if necessary. Identify by melting point 172 to 174°C and mixed melting point\*

Water layer  
Contains most of soluble soaps and split proteins

Colloidal Gold Solutions, Christina, V., and Green, C. S. Psychiatric Quart. July, 1928

The following reagents and method are recommended

**Conductivity Water** To every liter of distilled water (from a Stokes still) add 1 gm. of chromic acid and distill in an all glass distilling apparatus, avoiding use of rubber stoppers and connections. To every liter of water distilled over chromic acid add 1 gm. of barium hydrate. This may now be kept as a stock supply to be redistilled immediately before use.

**Reagents** Merek's gold chloride acid yellow, 1 per cent solution. Merek's potassium carbonate sp., 2 per cent solution. Merek's neutral formaldehyde solution, 1 to 40 dilution. These reagents are made up with fresh conductivity water.

**Preparation of Colloidal Gold** Heat 1 liter of fresh conductivity water to 60°C, at this temperature add 10 cc. of 1 per cent gold chloride solution and 8 cc. of 2 per cent potassium carbonate solution. Continue the heating until the temperature reaches 92° to 95°C, then remove the flame and add 5 cc. of formaldehyde solution, 1 to 40 dilution. Stir briskly until a full, bright red color develops.

## TITRATIONS

The permanent inorganic standards are prepared with slight modification, according to Taub. The following solutions are necessary for a  $P_H$  range of 6.6 to 7.4

N/2 Cobalt chloride	59.497 gm CoCl <sub>2</sub>	0.6H <sub>2</sub> O per liter of 1 per cent HCl
N/2 Ferric chloride	45.054 gm FeCl <sub>3</sub>	0.6H <sub>2</sub> O per liter of 1 per cent HCl

The indicator is prepared as follows. Dissolve 1 gm. of phenol red in 52 cc. of neutral 95 per cent alcohol, add 28.5 cc. of N/100 NaOH and sufficient conductivity water to bring the volume up to 250 cc. Store the indicator in a Pyrex bottle.

## MATCHING BLENDS

Table I indicates the amounts of chlorides necessary to give the correct colors for the standards.

TABLE I

$P_H$	CO (CC)	FE (CC)	CONDUCTIVITY H <sub>2</sub> O (CC)
6.6	1.8	6.5	11.7
6.7	2.1	5.95	11.95
6.8	2.4	5.4	12.2
6.9	2.9	4.15	12.9
7.0	3	2.9	13.0
7.1	4.5	2.1	13.65
7.2	5.0	1.5	13.7
7.3	6.1	0.4	13.1
7.4	7.2	0.3	12.5

The solutions are placed in test tubes of uniform length, diameter and thickness. They are then sealed into ampule form and sterilized in the autoclave at 15 pounds pressure for fifteen minutes to insure complete sterility. The tubes are allowed to cool overnight.

A comparator block is used to make the  $P_H$  determination. In making the determinations the test tubes should be of the same diameter and thickness as the uniform tubes used for standards.

To 10 cc. of the gold solution add 0.15 cc. of indicator and mix well. Place this tube in Hole E. In Hole B place a tube with distilled H<sub>2</sub>O. In Holes A, A<sub>1</sub>, place tubes containing gold solution without indicator. Holes C and D are for the  $P_H$  standards. If the gold solution does not match the 7.0  $P_H$  standard then N/100 NaOH or N/100 HCl is added, as the case may require. If the solution shows a  $P_H$  below 7.0 alkali is used for the titration, if it is above 7.0 then acid is employed. After each addition of acid or alkali to the gold solution the contents of the tube are thoroughly mixed by inverting the tube several times.

When the unknown gold solution is matched with the 7.0 standard the necessary correction is made for the entire volume of gold on hand.

After correction, test the neutrality of the solution by adding 17 cc. of 1 per cent NaCl to 5 cc. of the colloidal gold. The gold will be completely precipitated in an hour. It must give a typical curve with a kaawa parietic spinal fluid, and no reaction or a red blue change at the most in the first or second tube with a negative spinal fluid.

**LEPROSY** A Serological Method of Diagnosis Of Marchoux, E. and Caro, J. Ann. Inst. Pasteur 42 No. 5, 542 1928

Rubio's red corpuscle sedimentation reaction has been studied by these workers, who obtained positive results in half of ten cases by Rubio's technique in which 1 cc. of the patient's serum is mixed with 1 cc. of sheep's red corpuscles after washing them in normal saline and adding 10 per cent formal for twenty-four hours and then washing again. On keeping the mixture at 37°C sedimentation of the red corpuscles within an hour is a positive result. The present workers found that the reaction is a specific one, and that by adding five parts of serum to one of the sheep's corpuscles instead of equal parts, the reaction was positive in all their leprosy cases within half an hour.

In numerous new leper control cases the reaction was never positive. A temperature of 56° C for an hour destroys the complement of the serum, and one of 60° C destroys the specific substance as well.

**LEPROSY** The Staining of Leprosy Bacilli, Rudel, O. Centrbl f Bakteriol I Abt Orig 107 No 6, 357, 1928

The usual TB stain is most successful with leprosy bacilli when the solutions of stains and the sulphuric acid are more or less diluted.

The method is as follows:

(1) Carbol fuchsin (fuchsin 10, alcohol 100, concentrated carbolic acid 50 to 1000) with or without  $\frac{1}{4}$  to  $\frac{1}{2}$  water, heat until bubbles burst. (2) Wash several times with sulphuric acid (5 per cent) in water. (3) Wash with water (or in 70 per cent alcohol). (4) Loeffler's methylene blue (concentrated alcoholic methylene blue 300, caustic soda solution [0.01 - 100.0] 1000) in water, a few seconds up to one minute at the most. (5) Wash with water. (6) Dry and add Canada balsam (or instead of 6 rapidly wash in 70 per cent alcohol for two to five seconds absolute alcohol one to three seconds toluol balsam).

This stain is so characteristic for leprosy that it can almost be used for differential diagnosis against TB. The leprosy bacilli react much more easily to stains and acids than TB.

**TISSUE STAIN** Elastic Tissue Staining, French R W. Stain Tech 4 No 1 11, 1929

1 Bring sections affixed to the slide into water.

2 Stain in the modified Weigert elastic stain prepared as follows for one half to three hours:

Crystal violet	20 gm
Dextrin	0.5 gm
Resoreinol	40 gm
Water, distilled	2000 ml

Boil in a porcelain dish. When boiling briskly add 25 ml of 29 per cent aqueous ferric chloride, stir and continue boiling for 2 to 5 minutes. A heavy precipitate forms and the mixture assumes a greenish cast. Cool and filter. Boil the precipitate in 200 ml of 95 per cent ethyl alcohol over a water bath or electric hot plate. Cool, filter and make up to 200 ml. Add 4 ml of concentrated hydrochloric acid.

3 Differentiate in 95 per cent alcohol.

4 Wash in water.

As a routine procedure, preparations are then stained with the Weigert iron hematoxylin or Harris alum hematoxylin and counterstained with the Van Gieson mixture of picric acid and acid fuchsin mixture. Almost any desired stain may be used however, after the elastic stain. The staining is selective, only elastin being stained a brilliant yellowish green.

**TISSUE STAIN** Staining of Mitochondria and Bacteria in Plant Tissues, Dufrenoy, J. Stain Tech 4 No 1, 13, 1929

Milovidov, in Professor Guilhaumon's laboratory, has worked out the following technique to stain mitochondria red and bacteria blue in root nodules of legumes.

1 Kill tissues for twenty-four hours in a mixture of 1 per cent chromic acid (50 cc), 1 per cent potassium dichromate 50 cc, 40 per cent formalin (neutralized by adding powdered calcium carbonate) 8 cc.

2 Rinse twenty-four hours in running water, dehydrate, imbed, and cut very thin sections. (4)

3 Dissolve paraffin from slides, dip slides into very thin solution of collodion in absolute alcohol and ether. Run through series of alcohol and water.

- 4 Stain slides, at temperature of about 80 C in solution
 

Acid fuchsin	2 gm
Anilin water	10 gm
- 5 Wash in running water
- 6 Destain for a few seconds in
 

Aurantia	0.5 gm
70 per cent alcohol	100 gm
- 7 Wash in water
- 8 Treat for a few minutes in
 

Phosphomolybdic acid	1 gm
Sodium hydroxide 1 per cent solution	10 gm
Water	90 gm
- 9 Rinse in water

10 Stain for a few minutes in a solution of Unna's polychrome methylene blue diluted in three parts of water

#### STAIN Staining Methods for Bacteria and Yeasts Maneval W E Stain Tech 4 No 1 21 1929

For staining flagella of bacteria use actively motile organisms twenty to twenty four hours old allow to diffuse in sterile water twenty to thirty minutes transfer drop lets of the suspension to clean slides and let evaporate without spreading. Then treat two to four minutes with the following mordant tannic acid 10 to 20 per cent 50 cc ferric chloride 5 per cent 10 to 15 cc carbol fuchsin (Ziehl-Nielson) 5 cc hydrogen peroxide 3 per cent 6 to 8 cc Wash and stain two to three minutes with a mixture of basic fuchsin saturated alcoholic 10 cc anilin oil (1 part) and 95 per cent alcohol (3 parts) mixed 5 cc distilled water 30 cc acetic acid 4 per cent 1 cc Wash thoroughly with water

In some kinds of yeast the nucleus may be demonstrated by fixing smears with heat staining one minute with aqueous acid fuchsin followed by 5 per cent tannic acid for twenty seconds and finally washing with acidulated water

In staining the reaction may be varied by adding acid or alkali to the films and then dropping on the stain. A solution of acetic acid (1:250) or of sodium hydroxide (1:750) is satisfactory. One to four drops of the acid or 1 to 2 drops of alkali may be added to 6 drops of stain. An acid reaction is often the best and sometimes it is well to acidulate slightly the water used for washing

#### STAIN Preparation of Methylene Blue (Loeffler's) Conn, H. J. Stain Tech 4 No 1 27, 1929

The keeping qualities of Loeffler's methylene blue stain when made with commercial certified stains is improved by using the following formula for its preparation

Methylene blue medicinal (90 per cent dye content)	0.3 gm
Ethyl alcohol 95 per cent	30 cc

Dissolve and dilute in 100 cc of distilled water

#### TISSUE TECHNIC The Histological Demonstration of Adrenalin Baginski S Bull d'Histol et de Tech Micr 5 129 1928

Former methods for the demonstration of adrenalin in the tissues did not fix the tissues but tended to break them down. The following formulas act as fixatives for the tissue as well as reagents for the detection of adrenalin

Formula 1

2 per cent ammonium chromate	30 cc
1.25 per cent silver nitrate	20 cc
ammonia (0.912 sp gr)	3 to 4 drops

## Formula 2

2 per cent ammonium bichromate	50 cc
1.25 per cent silver nitrate	20 cc
ammonia (0.912 sp gr)	7 to 8 drops

These solutions keep well. Fix for three to twenty-four hours according to the size, but pieces should not be larger than 2 to 3 mm. Wash in distilled water until there is no more trace of chromium. Dehydrate slowly, clear in chloroform or xylol, not in cedar oil. Sections may be stained with various dyes. When natural adrenalin is diluted one to ten or fifteen million and is treated with these reagents a clear test for adrenalin is obtained in the form of a fine black precipitate.

**MILK** The Newman Combined Stain For Direct Counting of Bacteria In Milk  
Breed, R. S. Am J Pub Health 19 No 1 99 1929

The following formula extracts fat, fixes and stains

## FORMULA

Methylene blue powder	1 to 1.12 gm
Ethyl alcohol, 95 per cent	54 cc
Tetrachlorethane, technical	10 cc
Acetic acid, glacial	6 cc

Add alcohol to the tetrachlorethane in a flask and heat to a temperature not to exceed 70° C (if it is desired to use methyl alcohol, the temperature should not be raised to more than 55 to 60° C.)

Add the combined solution to the powdered methylene blue. Shake vigorously until the dye is completely dissolved. The original directions state that the glacial acetic acid should be added at this point. It is better to cool the solution before adding acid and the acid should be added very slowly. Further heating of the acid solution should be avoided. Filter and keep in a tightly stoppered bottle.

## DIRECTIONS FOR USE

- 1 Prepare the milk smear
- 2 When dry, immerse the smear in the solution and withdraw immediately
- 3 Drain until dry (thirty seconds)
- 4 Wash in water
- 5 Dry and observe

This solution permits much more rapid staining than does No 1 and yields a clear, well stained preparation. The smears readily wash off the slide unless they are dried before washing (Step No 3). If dried too rapidly the smears present a spongy appearance. If the smears are dipped in 70 per cent alcohol, this trouble may be prevented.

**CL BOTULINUS** Intramuscular Infection of Guinea Pigs With Spores Of Cl Botulinus,  
Coleman, G. E. Am J Hyg 9 No 1, 47, 1929

Coleman calls attention to the fact that animal inoculation with spores of Cl botulinus and Cl tetani is uniformly successful with the following technique.

Inoculations of 0.6 to 0.8 cc of freshly prepared 10 per cent solution of formalin are made into the leg muscle and a few hours later 1 cc of saline spore suspension is injected into the same spot.

## REVIEWS

Books for Review should be sent to Dr Warren T. Vaughn, Medical Arts Building  
Richmond, Va

### *Studies in Triesteza\**

THIS is a reprint from the Archiv fuer Schiffs- und Tropen Hygiene and represents a study made on sixteen year and a half old shorthorn steers in Argentina. The steers were infected with a condition known there as Triesteza which corresponds very closely to our Texas Fever. The study of the blood and of the reticuloendothelial system in various organs was quite thorough and it is illustrated with photographs and colored drawings. Some etiologic work and some cross immunity experiments were done and the author inclines to the view that the piroplasma and the malarial organisms are distinct because they have been separated as distinct and single infections in cattle.

### *The Methods Used in the Study of Hemolysis Including Hemagglutination†*

THIS is a reprint from Abderhalden's Handbook of Biological Methods and gives a very complete account of the biologic methods by which hemolysis can be produced, the animal and vegetable substances which are hemolytic and the immunologic significance of hemolysis.

### *A Collection of the Toxic and Lethal Doses for Laboratory Animals of the Most Common Poisons‡*

ABOUT 300 poisons are listed beginning with acetanilid and ending with zinc. The data are quite complete as in most cases not only is the dose given but also the animal, the method of administration, the result and the reference to the original article from which the data were obtained. Such a collection should be very valuable as a general guide or first approximation to the dose but they state that pharmacologists will understand that all such data are more or less variable and should not be used until checked on one's own animals and working with one's own methods and technique.

### *Combating Loss of Blood by Transfusion and Filling the Vessels§*

THIS is one of the Monographic Therapy series and treats the subject in a very practical manner. In the transfusion of homologous blood its scientific basis is discussed, the methods of matching the bloods are given and the technique of the transfusion itself. Then follows a discussion of the indications and the results to be expected.

The clinical justification for filling the blood vessels with fluids other than blood is given, the various fluids that may be so used are described and their practical application is discussed. He strongly urges the use of Ringer's solution rather than plain physiologic salt solution.

*Studies in Triesteza (Piroplasmosis and Anaplasmosis of Cattle)* By Felipe Jimenez d'Asua, Roberto L. D'Elia, Juan A. Zuccarini and Miguel J. Kuhn. Published by Johann Ambrosius Barth, Leipzig, 1928.

*The Methods Used in the Study of Hemolysis Including Hemagglutination* By Hans Sachs and Alfred Klopstock. Published by Urban and Schwarzenberg, Berlin and Vienna, 1928.

*A Collection of the Toxic and Lethal Doses for Laboratory Animals of the Most Common Poisons* By E. Flury and Z. Frick. This is Liefte no. 1 of Abderhalden's Handbuch der Biologischen Arbeitsmethoden. Vol. IV. Teil 1 and Heft 1. Published by Urban and Schwarzenberg, Berlin, 1928.

*Combating Loss of Blood by Transfusion and Filling the Vessels* By Clairmont von den Velden and Wolff. Published by Georg Thieme, Leipzig, 1928.

NOTE. In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

### *Handbuch der biologischen Arbeitsmethoden*<sup>\*</sup>

TWO more parts of this monumental work which is typically German in thoroughness, completeness, and in the excellence of its execution. In no other country could a publisher hope to make his expenses on such an undertaking. Even in rich America the National Research Council had to pass the hat and take up a collection before it could get a publisher for its International Critical Tables. Yet Urban and Schwarzenberg issue this *Handbuch* along with their regular work and find enough purchasers to make it profitable.

It is this wide spread spirit of thoroughness that makes Germany such a difficult competitor.

Part 238 contains articles by C. H. Best of Toronto on the Preparation of Insulin and on the Standardization of Insulin. It also contains an elaborate article on the Methods for the Experimental and Histological Examination of the Testis by Schinz and Slotopolsky and one on the Physiological Assay of Cholin by J. W. Lelieur.

Part 235 contains the following articles: The Photomicrographic Method of Bethe Hæppel by Paul Hæppel, Methods for the Investigation of and Changes in Irritability and Conductivity in Electrotonus by Otto Weis, Methods for Studying the Physiology of Work with Special Reference to Industrial Fatigue by Edgar Atzler, and Methods for Determining the Distribution of Body Weight on the Different Parts of the Sole of the Foot by Adolph Basler.

### *Alcohol-Extract-Reaction, A-E-R, for the Diagnosis of Pregnancy, Cancer, and the Sex of the Unborn Child*<sup>†</sup>

THIS is a book of 260 pages on a modification of the Abderhalden reaction. Instead of allowing the patient's serum and tissue to interact the authors mix the serum with an extract of the tissue, instead of dialysing out the digested products the authors remove the undigested proteins by adding 96 per cent alcohol. They claim that these are distinct improvements but admit frankly that they are on their way and have not yet arrived at the goal they have in mind which is to devise diagnostic laboratory tests for many other conditions which will be as reliable as is the Wassermann test in syphilis.

### *Die Serodiagnose der Tuberculose*<sup>‡</sup>

PINNER gives directions for and discusses agglutination, precipitation, complement fixation, opsonins, the microstigmata reaction, bacteriolysis, sedimentation of red blood corpuscles, and flocculation reactions as applied to the diagnosis of tuberculosis. He considers complement fixation by far the best specific method but the difficulty with all of them is the inability to distinguish between an active tuberculous process and one that is latent or quiescent.

### *Proceedings of the Berlin Medical Society for the Year 1926*<sup>§</sup>

THIS is a stately volume of nearly 300 pages and contains a list of their more than 1700 members with addresses, the minutes of their meetings including the annual reports from which it appears that they have a library of 52,000 volumes and take 200 periodicals, and the full text of all of the papers presented at their meetings together with the discussions which followed. Several of the articles are illustrated with special plates.

<sup>\*</sup>Handbuch der biologischen Arbeitsmethoden. Lieferung 235 and 238.

<sup>†</sup>Alcohol-Extract-Reaction, A-E-R, for the Diagnosis of Pregnancy, Cancer, and the Sex of the Unborn Child. By W. Luetjge and W. von Mertz. S. Hitzel Leipzig 1927.

<sup>‡</sup>Die Serodiagnose der Tuberculose (Volume 28 of the Library of Tuberculosis being published by Dr. Lydia Rabinowitsch.) By Max Pinner, Director of the State Tuberculosis Sanatorium (Spring Hills Sanatorium) at Northville, Michigan, U. S. A.

<sup>§</sup>Proceedings of the Berlin Medical Society for the year 1926. Published by the Society, Urban and Schwarzenberg, Berlin.

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## EDITORIALS

### Lead in the Treatment of Cancer

THE search for new and better weapons for use in the treatment of disease is indeed a slow and plodding one. Every few years a discovery of the first order is recorded. None would deny a place in this category to insulin or to the liver treatment of pernicious anemia. For each of these outstanding therapeutic advances how many are there which have been hailed as great discoveries but which have not stood the test of prolonged therapeutic trial? How many such have fallen entirely into the discard? How many have temporarily passed into oblivion to be rediscovered after the passage of years and perhaps with slightly improved technique, have been found after all to possess definite value?

Three years ago the lead treatment of cancer was hailed as possessing great promise. In the interval it has been subjected both to therapeutic trial and to close laboratory experimental study. We are not yet in a position to



assay its proper value but it is interesting after the lapse of three years to survey the progress or lack of progress made

By a coincidence two communications have appeared almost simultaneously which together bring the literature on the lead treatment of cancer and incidentally our knowledge of the subject up to-date. The first, a review of the *Present State of the Treatment of Malignant Tumors with Lead* by Jacques Lavedan, Director of Laboratory at the Radium Institute of the University of Paris correlates the available literature. The second, by W. Blair Bell entitled *The Present Position of Lead Therapy in Malignant Disease* summarizes the work of its discoverer up to the present.

Research has been carried on both in the laboratory and in the clinic. In both instances, particularly the second, comparison of results observed by different workers is difficult because in most cases details or even major factors in the technique have varied with nearly each observer. In the clinic, for example, the highly toxic effect of so called colloidal lead has been recognized and attempts have been made to produce lead compounds of therapeutic potency equal to that claimed for the original used by Blair Bell but with reduced toxicity. As a consequence a relatively large number of compounds of lead have been employed and results with one compound cannot justly be compared with results from another.

In addition many cases have been treated not with lead at all but with a combination of lead and x-ray or lead and radium therapy. Furthermore some have been subjected to prior surgery while others have not and some cases of cancer have been treated while at a relatively early stage of evolution while others have only been subjected to lead therapy if considered hopelessly incurable.

The clinical reports may be roughly divided into two groups, those favorable and those unfavorable. Favorable reports should not necessarily predicate an actual cure in a high percentage of cases but do recognize indisputable benefit attributable to lead therapy in a proportion, and sometimes apparent cure in a few.

Fitzwilliams using a lead preparation different from that originally proposed by Blair Bell has treated 60 cases most of which have also been subjected to surgery. He reports encouraging results especially in several cancers of the esophagus. Carver and Stone have reported results in 21 cases, some treated with lead alone and others with lead and x-ray therapy. In seven cases of cancer of the breast the tumor diminished markedly in size and in two they felt justified in describing clinical cure. They feel that in osteosarcoma combined lead and ray therapy appears to be the method of choice. Oldham has reported a clinical cure after eighteen months, of a mixed cell sarcoma of the jaw, with glandular metastases. This case was treated with surgery and lead.

Loewy and Loiseleur treated a series of hopeless cancers with a lead preparation of their own with very promising results. When, however, they applied the same therapy to a series of less advanced cases the results were not as good. However, even here 4 out of 21 cases appeared to have been arrested. Thomson applied lead therapy to 55 cancer patients between

October, 1926, and June, 1928. He used three different compounds of lead one of which was the original Bell compound. He found this to be the more effective. Of the 55, fifteen were definitely improved, at least temporarily, of whom 3 have shown no further growth and two are considered cured. Ullman like the others reports cases which have shown no benefit and others which have. Some have been treated with lead alone and others with lead and radiation. Of 15 cancers of the breast 8 showed incontestable improvement of longer or shorter duration. Improvement was also seen in 3 of 4 cancers of the uterus, 2 out of 3 cancers of the maxilla, 2 of 3 cancers of the prostate. Souland, Costelow and Maitland have treated 31 cancers with combined lead and radiation. They described no cures but in 4 while the tumor had not disappeared its further development appeared to be arrested. They feel that they have evidence of at least temporary beneficial effect. However here as elsewhere the problem arises as to how much is to be attributed to ray therapy and how much to lead.

Knox who has tried both lead therapy and combined lead and ray therapy feels that the latter is of distinctly superior value. He presents a series of 40 cases several of whom showed temporary improvement and 4 of whom may be described as clinically cured. Of course in these clinical cures in all the series mentioned the time interval is too short to say there will be no recurrence.

Finally we have to report among the communications presenting evidence of some beneficial effect from lead treatment the recent communication from Blair Bell mentioned above covering the results observed in the Liverpool Medical Research Organization. Five hundred and sixty six cancerous individuals were treated between November 9 1920 and November 9 1928. Many of these died before the completion of treatment but of the total, 303 cases received more than one half of the minimum therapeutic course recommended. 77 died after full treatment, 7 died of intercurrent infection while under treatment, 161 died of the disease before treatment could be completed. 5 died as a result of the extensive destruction from the disease. Twenty two refused to complete the treatment. In 31 treatment has been completed too recently for the results to be estimated.

Two are believed to have been cured but died from other affections (apoplexy, and toxemia of pregnancy). In 12 the disease has been completely arrested and in 51 the patients are believed cured and the treatment has been discontinued. The last three classifications total 65 cases and Bell records these as successes. He concludes that of 303 cases who received more than half the minimum course advised, 65 or 21.5 per cent resulted successfully.

Opposed to this series of reports we find a sufficiently large number of investigators who can attribute no value to lead therapy. Wyard of the London Cancer Hospital found evidence of benefit in only one of 40 cases treated. This one case presented an enlarged infraclavicular gland following a breast amputation. Under lead treatment the adenopathy disappeared but unfortunately its nature was not definitely known, no biopsy having been made. Wyard believes that lead is entirely without value in the treatment of malignant tumors.

Hume finds no evidence that intravenous injection of lead caused destruction of cancer tissue. Indeed, he feels that this treatment hastened

death. He finds no difference histologically in human malignant tumors before and after lead injection.

Simpson concludes from his observations that lead does not provide the solution of the therapeutic problem of cancer. Of 19 cases only one has survived and this was a cancer of the breast removed as thoroughly as possible by surgery before the institution of lead therapy.

Waters, Colston, and Gay have treated 7 inoperable carcinomas with combined ray and lead therapy. Five are dead and the sixth a case of carcinoma of the pleura improved but developed a pulmonary metastasis, and the seventh with cancer of the breast and metastasis, is improved. In a second series of seven, 3 are definitely improved and 4 are dead. The authors conclude that benefit was attributable in these cases to ray therapy rather than lead since injection of the metal did not prevent the occurrence of metastases.

Leaving for a moment this division of opinion concerning the clinical value of lead, we find some most interesting experimental observations. Mottram finds that in experimental mouse tumor in which the course is quite constant, the lethal dose of lead is accurately known and the minimum curative dose of radium is known. Combined radiation and lead treatment produced cures when the dose of each of the two therapeutic measures was distinctly below the minimum curative dose for each used alone. The combination of the two forms of treatment, each insufficient when used alone, resulted in cure. Wood likewise believes that lead and radiation possess synergistic action.

Simpson working with experimental mouse tumors found no curative effect from colloidal lead alone. In these animals at necropsy he was unable to demonstrate lead in the tumor either by microscope examination or spectroscopic analysis. Bang also found no curative action, indeed not even a perceptible delay in the evolution of the mouse tumor following lead treatment of stated dosage. His dosages were small.

However, as Wood brings out, the fact that lead shows no evidence of therapeutic action on certain animal tumors does not show conclusively that it is not of value in the treatment of human cancer. The evolution of malignant neoplasm is not the same in the two groups.

The observation of Simpson that lead does not accumulate in malignant tumors in experimental animals deserves more attention. It will be recalled that Blair Bell's original concept was that malignant cells possess a very close biologic relationship to the embryonal cells of the chorionic villi, and that just as abortion in lead poisoning is due to a specific affinity between these embryonal cells and the lead, the results of lead in cancer therapy are due to an affinity between the metal and the cancer cells. Simpson found no definite evidence of lead accumulation in mouse tumor. Thomson has found that the spleen and the liver possess a decidedly greater affinity for lead than does the tumor itself.

Soiland and his associates find that colloidal lead does not remain long in the circulating blood. It is quickly phagocytized and becomes fixed in the reticuloendothelial system, accumulating in the bone, liver, and spleen. As long as it remains in a stable state it is held by the histiocytes outside the

tumor After two or three months it becomes oxidized and hydrolyzed and is eliminated chiefly in the bile Chemically the major portion is recovered from the spleen, liver, and bone Both primary and metastatic tumors contain little or none

Loewy and Loiseleur using oxide of lead which in contrast to the particles of colloidal lead may enter into immediate combination weighed the lead present in a breast cancer removed forty eight hours after an injection of one hundred mg They showed the presence of 0.94 mg in the 16 gm of malignant tissue Here then was actual fixation of lead in the tumor but in only a small fraction of the amount introduced

Glynn found histologic evidence that lead had influenced the tumor cells in only a small proportion of tumors Fry concludes again from histologic study that lead produces changes in the convoluted tubules of the kidney and a fatty degeneration of the liver but he was unable to find any evidence of histologic changes in the cancer cells themselves

Returning for a moment to Bell's theory of resemblance to chorionic cells, it is of interest to note that in chorioepitheliomas, malignant tumors arising from the chorion both Knox and Stone and Cramer were unable to observe any favorable influence following lead treatment

Apparently lead if it does exert an action, does not do so by direct effect on the malignant cells How then does it appear to act?

Lavedan of the Radium Institute of the University of Paris among others confirms the original observation of Blau Bell that lead therapy produces local changes in the tumor such as pain and swelling If it does not exert a direct action on the malignant cells what then is its *modus operandi*? In Wood's experimental work on rats he observed the same congestion and edema in the tumor He ascribes it to vascular thrombosis with resulting necrosis The action then would appear to be vascular with only a secondary necrosis of the tissues and cancer cells Martland Sochoky and Hoffmann also describe peripheral vascular thrombosis with subsequent necrosis of the neoplasm Lavedan describes a carcinoma of the tongue in which following combined radium and lead therapy the tongue necrosed and sloughed off entirely, without the loss of a drop of blood Then radium technique was standard and they had had no such experience with radium alone They interpret this as demonstrating the thrombosing action of lead

What shall we say, after three or more years of careful study in the hands of many observers, is the present status of the lead treatment of cancer? We can safely say that in a minority of cases there has been evidence of clinical improvement and in a few there appears to have been actual cure These more or less promising results have almost without exception been obtained in cases which had already advanced to the stage of being considered hopelessly incurable We can gather from the preceding review that it is not at all certain that cases treated either would necessarily show better results The preponderance of experimental evidence as we have it so far is that lead acts nonspecifically It acts not through a fatal toxic affinity for cancer cells but by interfering with the circulation of blood and lymph through the tumor thereby strangling the new growth



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## ON TO PORTLAND—EIGHTH ANNUAL CONVENTION

### American Society of Clinical Pathologists

**A** GAIN the members of the American Society of Clinical Pathologists are looking forward with pleasant anticipation to the next meeting of the organization which is to be held in Portland Oregon July 5 6, and 8 1929 With each succeeding year the Fellows of the Society are being bound more closely together by scientific as well as sentimental ties The friendships formed and strengthened at the conventions are a source of solace and cheer in the trials and tribulations of the clinical pathologist in his struggle against adverse conditions The conventions tend to confirm the conviction of the clinical pathologist that our specialty has now become firmly rooted in the medical world, particularly in the hospital field We find that a better day is dawning for the man who has prepared himself by proper grounding in pathology, bacteriology, biochemistry, and allied sciences and thus made himself indispensable to the clinician both in private as well as institutional practice Attendance at the conventions is also an inspiration from the scientific standpoint The papers stimulate the Fellows to better efforts in self improvement

The program this year bids fair to equal if not surpass the brilliant successes of the previous years The next convention marks the eighth mile



DR J H BLACK  
Dallas, Texas  
President Elect



DR CHARLES R DRAKE  
Minneapolis, Minn  
Vice President



DR H J CORPER  
Denver, Colo  
Secretary Treasurer

stone in the existence of our organization which first saw the light of day in St. Louis, in 1922. Little did the pioneers dream that that small band of Charter Members would swell to a membership of now close to four hundred, numbering among them men who have distinguished themselves by original scientific work in the various branches of clinical pathology. A perusal of the tentative program printed on another page shows the diversity of scientific work, almost encyclopedic in its scope which comes within the purview of the clinical pathologist.

A half day will be devoted to the discussion of a very timely subject in scientific circles, that of undulant fever on which a number of men who have had experience with this new disease will speak, giving the results of their



MOUNT RAINIER

investigations. There is also a paper on Tularemia by Dr. Walter M. Simpson. Our past president, Dr. Wm. G. Eaton, who has never missed a meeting, will give us further information on his studies in the colorimetric methods of determination of urinary constituents. Equally interesting will be further studies by Drs. A. H. Sanford and Charles Sheard on the determination of hemoglobin by a photoelectric method. Those interested in pathologic anatomy and in protozoology will likewise find titles of papers which they would like to hear.

The Committee on the Registration of Technicians will have an interesting report to present. Over four hundred laboratory workers throughout the





DR A H SANFORD  
Rochester, Minn  
Chairman, Executive  
Committee  
Past President,  
1927 1928



DR W W COULTER  
Houston, Texas  
Executive Committee



DR FREDERIC E SONDRÉN  
New York, N Y  
Executive Committee  
Past President 1925 1926



DR HERMAN SPITZ  
Nashville, Tenn  
Executive Committee



DR WILLIAM G EATON  
Newark, N J  
Executive Committee  
Past President  
1926 1927

United States and its possessions as well as Canada have enthusiastically endorsed the Registry and have signified their intention to register by filling out the official application for certificates of approval

One of the features of the meeting is the Round Table Discussion on economic problems where opportunity is given all the members present to let off steam and get the grouse out of their system, receiving in return stimulus and encouragement as well as useful hints from fellow members. Considerable good has already been effected by these informal discussions. They are a source of great comfort and good fellowship to all the participants.

The place of the Convention on the West Coast need not deter anyone from attending. Aside from a patriotic duty to See America First, the members and their wives will enjoy the outing to the Pacific Coast, which will afford them facilities of viewing the wonderful scene places at their destina-



ICE CAVE IN MT. RAINIER NATIONAL PARK

tion and en route. What convention city can be more alluring than Portland, giving an opportunity to visit Mt. Rainier National Park, the unsurpassed scenic beauties of Yellowstone and the Rocky Mountains. The latter are of particular importance as within their confines is situated the City of Denver where the headquarters of the American Society of Clinical Pathologists is located. The writer succumbs to the temptation of doing a little boosting on his own account by including the Rocky Mountains on the itinerary. A cordial invitation is extended to all the Fellows and their families to visit Denver in their travels, either coming or going.

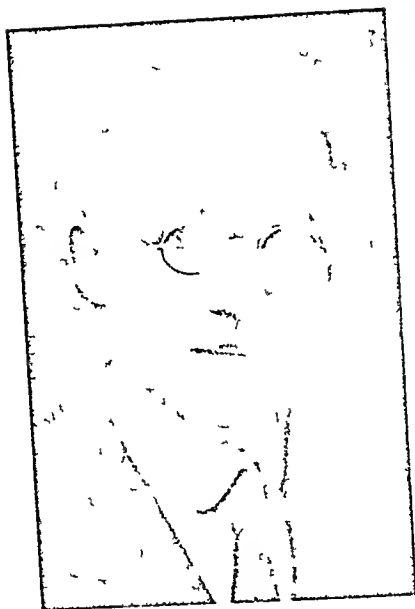
You are therefore urged to throw all cares aside and rejuvenate yourself with an injection of Western Hospitality and a wonderful vacation by attending the Eighth Annual Convention of the American Society of Clinical Pathologists.



DR C H MANLOVE  
Portland, Oregon  
Chairman, Board of  
Censors  
Chairman, Committee  
on Exhibits



DR ROBERT A KILDUFF  
Atlantic City, N J  
Board of Censors  
Chairman Program Committee



DR JOHN A KOLM  
Philadelphia, Pa  
Chairman, Publication Committee  
Executive Committee  
Past President, 1921 1925



DR H R MILLS  
Tampa, Florida  
Board of Censors



DR RUBEN OTT  
New York, N Y  
Board of Censors

# American Society of Clinical Pathologists

## Tentative Program

### Of the Eighth Annual Convention

Portland Oregon

July 5 6 and 8 1929

FRIDAY JULY 5 1929 9 A M

#### Scientific Program

- The Effect of the Presence of Bile on the Agglutination Reaction By Ruth Gilbert, M D  
and Marian B Coleman B S, Albany N Y
- Tularemia (Francis Disease) A Report of Ten New Cases with an Analysis of Sixty Three  
Cases Occurring in Dayton Ohio By Walter M Simpson M D Dayton Ohio
- The Routine Use of the Photoelectric Hemoglobinometer By A H Sanford M D and  
Charles Sheard Ph D Rochester Minn
- Polyerthemia (Patent Foramen Ovale) By Herman Spitz M D Nashville Tenn
- Acute Diffuse Myelitis Following Intravenous Injection of Arsphenamine By Ernest  
Scott, M D, and H L Reinhart M D Columbus, Ohio
- Improved Colorimetric Procedures for the Quantitative Estimation of the Proteins of the  
Cerebrospinal Fluid By Philip B Matz M D, and Nathan Novick Washington  
D C
- Pachymeningitis Hemorrhagica Interna By Frederick H Lamb M D Davenport Iowa

FRIDAY JULY 5 1929 2 P M

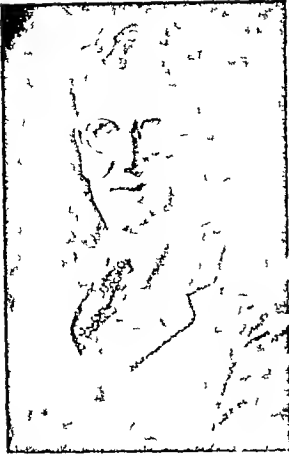
#### Symposium on Undulant Fever

- Undulant Fever in Man A Clinical Analysis of Thirty Three Cases By A S Giordano  
M D and R L Sensesch M D South Bend Ind
- Discussion on Undulant Fever By Walter M Simpson M D Dayton Ohio
- The Pathogenicity for Monkeys of Brucella Abortus By Merrill J King M D Mount  
McGregor N Y
- The Etiology and Diagnosis of Undulant Fever in the United States By Charles M  
Carpenter M D, and Ruth Book Ph D Ithaca N Y
- Bacteriology of Undulant Fever By K J Meyer M D San Francisco Calif to be read  
by J C Geiger M D San Francisco Calif

FRIDAY JULY 5 1929 4 P M

#### Round Table Discussion

- Virtuosity in Clinical Pathology By Philip Halkowitz M D Denver, Colorado
- Problems By W G Gamble Jr Chicago Illinois
- The Hospital Situation (a) Economic (b) Scientific (c) Statistical, (d) Relation to  
American College of Surgeons
- Postmortems
- The Intern Question
- Is the Cost of Laboratory Work Too High?
- The Clinical Pathologist in the Rural Hospital



DR ERNEST SCOTT  
Columbus, Ohio  
Board of Censors



DR B W RHAMA  
Fort Wayne, Ind  
Board of Censors



DR T B MAGATH  
Rochester, Minn  
Editor in Chief



DR ROBERT A KEILTY  
Washington, D C  
Associate Editor



DR KENNETH M LYNCH  
Charleston, S C  
Associate Editor

## SATURDAY JULY 6 1929 9 A M

- Gingivitis V The Character of the Exudate in Gingivitis By Robert A Keilty MD  
Washington, D C
- The Tuberculous Cavity By Alfred Blumberg MD Oteen N C
- Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of  
Acid fast Nonpathogens on Different Media with Their Reaction to Chemical  
Reagents By H J Corper MD and Arto Uusi PhD Denver Colorado
- A Recently Isolated Bacillus of the Hemophilic Group By F W Hartman MD and  
Edna Jackson M S Detroit Michigan
- Milk Borne Rabies By F R Murrage MD Denver Colorado
- Observations on Intestinal Protozoosis By Rawson J Lickard MD San Diego Calif
- Case of Agnucleate Angina By Herman C Beck MD Richmond Virginia

## SATURDAY JULY 6 1929 2 P M

- Pathology of the Petrosal Endothelial System By Zera F Bolin MD San Francisco  
California
- Reticulocytes Their Identification and Significance By C L Spolir MD and Alice  
Bustine Columbus Ohio
- Quantitative Microscopic Urinalysis By Wm C Exton MD Newark N J
- New Quantitative Clinical Methods for the Junior Scopometer (1) Protein in Urine (2)  
Protein in Blood (3) Protein in Spinal Fluid (4) Globulin in Urine (5) Sugar  
in Urine (6) Sugar in Blood (7) Urea in Urine (8) Ammonia in Urine (9)  
Creatinine in Urine (10) Sulphur Partition in Urine By Wm G Exton MD  
Anton R Rose PhD Fred Schattner PhD and P V Wells DSc Newark N  
J (To be read by title)
- Embryonal Carcinoma of the Testis By J W Larson MD Bismarck N D
- Malignant Tumors of the Testis By O A Brines MD Detroit Michigan

## SATURDAY JULY 6 1929 7 P M

## Annual Banquet

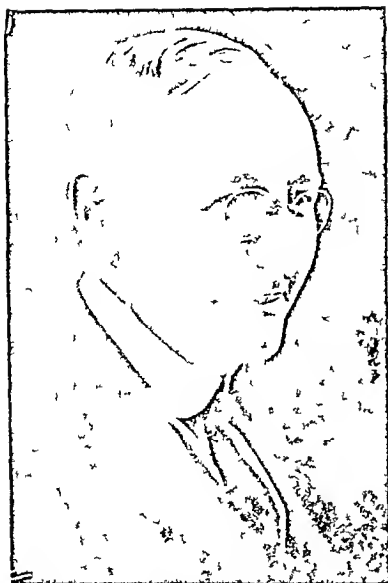
- Presidential Address By Dr Frank W Hartmann Detroit Michigan
- Address By Dr Richard B Dillehunt Dean of the Medical School of the University of  
Oregon Portland Oregon
- Address By Dr Cyrus C Sturgis Professor of Medicine at the University of Michigan  
Ann Arbor Michigan
- Presentation of the Ward Burdick Research Award

## MONDAY JULY 8 1929 9 A M TO 12 AND 2 P M TO 5 P M

## Business Session

- Call to Order
- Reading of Minutes
- Unfinished Business
- Reports of Committees

- Executive Committee*—Dr A H Sanford Chairman Rochester Minn
- Publication Committee*—Dr John A Kolmer Chairman Philadelphia Pa
- Editorial Committee*—Dr T B Magath Editor in Chief Rochester Minn
- Committee on Registration of Technicians*—Dr Philip Hilkowitz Chairman Denver  
Colo
- Public Relations Committee*—Dr Edward F Cooke Chairman Houston Texas
- Service Bureau Committee*—Dr H J Corper Chairman Denver Colorado
- Research Committee*—Dr Alvin G Foord Chairman Buffalo N Y
- Committee on Exhibits*—Dr C H Manlove Chairman Portland Oregon
- Committee on Nomenclature*—Dr I H Black Chairman Dallas Texas



DR. PHIL HILLERWITZ  
Denver, Colo  
Chairman, Committee on Registration  
of Technicians  
Past President, 1922-1923



DR. WM. CARPENTER MACCARTHY  
Rochester, Minn  
Past President, 1923-1924



DR. ALVIN G. FOORD  
Buffalo, N. Y.  
Chairman, Research  
Committee



DR. H. H. FOSKETT  
Portland, Oregon  
Chairman, Local  
Arrangements Committee

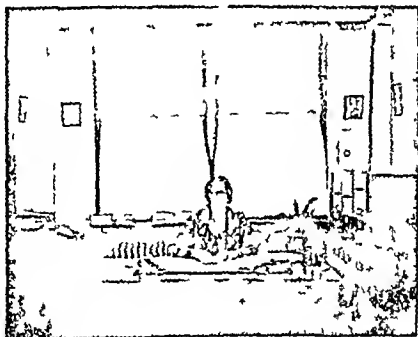


DR. EDWARD F. COONF  
Houston, Texas  
Chairman, Public  
Relations Committee

Report of Board of Censors—Election of New Members  
 New Business  
 Report of Nominating Committee—Nomination of Officers  
 Election of Officers  
 Induction of Officers  
 Adjournment

### Office and Headquarters

The office and headquarters of the American Society of Clinical Pathologists was moved during the year to a downtown location in one of the medical office buildings where the close association with clinicians as well as the facilities of the medical library make the location a very desirable one from many standpoints. We reproduce herewith photographs of the interior with the assistant to the Secretary at her desk.



OFFICE OF THE SECRETARY FRONT VIEW

An elaborate system of filing devices is part of the equipment, in order to keep in touch with our members as well as to house the data pertaining to the Registry of Technicians.

The map shown in one of the pictures gives a graphic demonstration of the geographic location of our members. The dark pins show the clinical pathologists who are not yet members.

The walls are decorated with photographs of past presidents of the Society.

The new location of our headquarters has added greatly to the efficiency of the Secretariat, being more accessible to the Secretary as well as to the Chairman of the Registry of Technicians, permitting them to spend more time in the office than under the old arrangement in a private hospital. The more prompt mail delivery is also a factor in the increased efficiency. On account of the semipublic character of our work and its benefits to the medical profession the administrators of the building are charging only a nominal rental.



All Clinical Pathologists and licensees of the Registry are invited to visit our office and become personally acquainted. A most cordial invitation is extended to members to stop over on their way to the meeting in Portland to visit our headquarters where a cheerful welcome awaits them. Facilities will



OFFICE OF THE SECRETARY, SIDE VIEW

be afforded for seeing the wonderful sights of the city as well as the mountains in the environs. The local members will be pleased to extend hospitality to our colleagues and their wives who stop off in Denver going to or coming from the convention.

# The Journal of Laboratory and Clinical Medicine

VOL XIV

ST LOUIS MO JUNE 1929

No 9

## CLINICAL AND EXPERIMENTAL

### A GROUP OF HIGHER BACTERIA FROM THE GENITOURINARY TRACT\*

#### I. PRELIMINARY COMMUNICATION

By SARA A. SCUDLER, B. A. AND DAVID L. BELDING, M. D. BOSTON, MASS.

THE incidence of nongonorrheal infections of the genitourinary tract is undoubtedly greater than ordinarily believed but the difficulty of distinguishing between primary and secondary invaders renders the statistics unreliable. The common pus-forming bacilli and cocci have been reported in numerous cases but the higher bacteria have not usually been associated with chronic irritation of the genitourinary tract.

Three strains of higher bacteria have been isolated from the genitourinary tract: strain A from the cervix of a child, strains B and C from the prostatic secretions of patients who had clinical signs of chronic urethritis but no positive clinical or laboratory evidence of gonorrhea. The morphologic and biologic characteristics of these strains are recorded in order to facilitate their identification and later classification, since there is at present no universal agreement as to a satisfactory differentiation between the orders of higher bacteria. Their association with chronic irritation of the genitourinary tract and their superficial resemblance to streptococci make them of clinical as well as bacteriologic interest.

#### TECHNICAL METHODS

*Clinical Material*.—In obtaining the original cultures from the child plates were streaked with secretions directly from the upper vagina and cervix. The cultures from the adults were made from fresh prostatic fluid kept at body

From the Evans Memorial, Boston. Manuscript received for publication August 1928.

temperature until centrifugation. The medium on which the strains were isolated was veal infusion agar adapted by enrichment for the isolation of the gonococcus. In each instance the colonies on the original plates were numerous, plaque-like, and from pin-point to 10 mm in size. Technical details have been previously described by Seudder.

**CASE A**—This strain was isolated from a five year old girl who had been under observation since the age of four months with a diagnosis of malnutrition and rickets. At the age of seven months she received the additional diagnosis of gonorrheal vaginitis and during the next three years positive gonococcus smears were obtained from time to time. Between the ages of three and one half and five and one half years she was persistently examined for gonococci as a research case. During this period she had a mild intermittent inflammation of the urethra, cervix, and vagina, accompanied at times by a slight watery discharge. Smears showed consistently a lancet shaped gram positive diplococcus in pairs and short chains with rare pus cells, even when the clinical signs were least suggestive of gonorrhea. At times a gram negative intracellular diplococcus, morphologically like the gonococcus was also present. Repeated cultures failed to yield gonococci. At the age of five a provocative injection of gonococcus vaccine slightly increased the clinical signs of inflammation and the smears from the cervix showed both gram negative and gram positive diplococci, but cultures yielded only pin point colonies which proved to be a pleomorphic bacterium, Strain A.

**CASE B**—A young man of twenty six years with a history of repeated attacks of tonsillitis, was exposed to venereal infection eleven months previous to culturing. One month later he was operated upon for simple mastoid. Subsequently he noticed a scalding upon urination which was relieved by urotropin. Five months later a diagnosis of chronic urethritis, probably gonorrheal, was made. Occasional shreds in the urine and a morning drop were present, but there was never a definite discharge. Repeated smear examinations from the urethra and prostatic secretion failed to show gonococci. Cultures taken from a slight secretion obtained by prostatic massage gave no gonococci, a few colonies of staphylococci, and numerous delicate pin point colonies of a pleomorphic chain organism, Strain B. Direct smears from the secretion showed pus cells and various gram positive cocci with a few gram negative diplococci.

**CASE C**—A male patient, forty eight years old, had a persistent intermittent discharge since an attack of gonorrhea twenty eight years ago. An examination ten years ago revealed increased massage fluid and prostatic plugs, while smears showed numerous pus cells and a variety of unidentified organisms. During the last ten years no gonococci have been found in some forty examinations of the prostatic fluid, all of which were essentially the same, except for a gradual diminution of the amount of pus during treatment. At the time of isolation of Strain C the direct smear showed 50 per cent pus cells, many spermatozoa, and coccoid organisms in chains. In addition some of the leucocytes contained gram negative bacteria morphologically resembling gonococci. Twenty four to seventy two hour incubation of streaked plates yielded only pleomorphic chain cocci in pure culture, which on further cultivation in broth proved to be higher bacteria.

*Laboratory Methods*—Culture media of the hormone type were prepared with a minimum degree of heating and filtering. Adjustment was made with 10 per cent sodium carbonate, and the reaction was determined by means of Clark and Lub's technique with Sorensen's standard phosphates. The hormone media were used in the form of broth, 0.5 per cent agar and 1.5 per cent agar with and without defibrinated blood or other enrichment. Comparative fermentation tests, using nonsol glass tubes, were made with the following culture media: (1) hormone 0.5 per cent sugar free agar, (2) 0.5 per cent agar with peptone and standard amounts of sodium monohydrogen phosphate and potas-

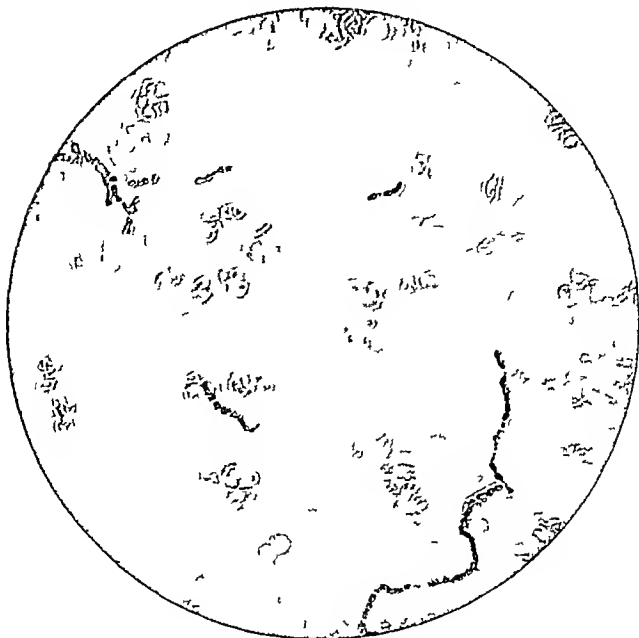


PLATE I FIG 1

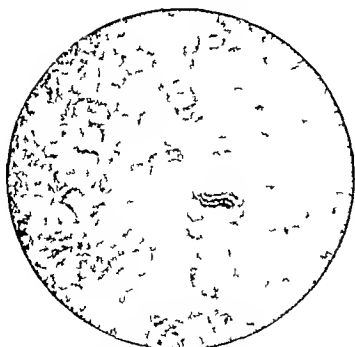


PLATE I FIG 2

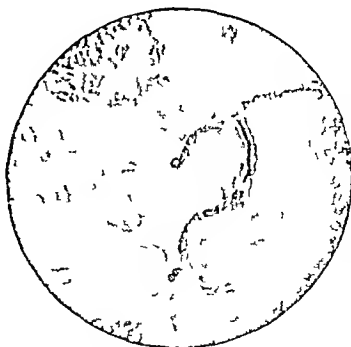


PLATE I FIG 3

Sperm culture of strain C four hours at 37 C

Fig 1 Sheathed filament and lancet shaped forms

Fig & 3 Filaments which show dichotomous branching



PLATE II, FIG 1



PLATE II, FIG 2

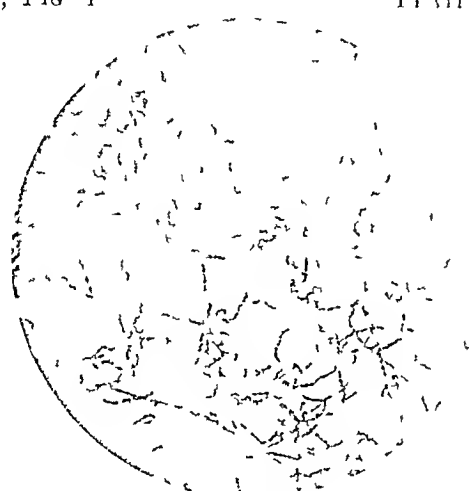


PLATE II FIG 3



PLATE II FIG 4

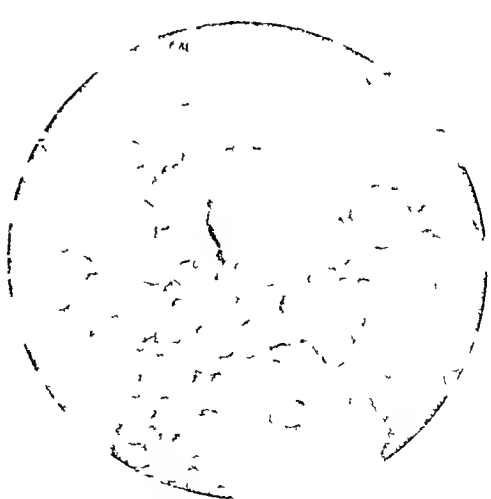


PLATE II, FIG 5

Fig 1 4 Twenty four hour artificial cultures  
 1 In water of condensation on yeast agar slant 2 and 4 in semi-solid agar 3 in  
 vitamine broth marked parallel arrangement  
 5 Thread form in four hour sperm culture

sium dihydrogen phosphate, and (3) potassium monohydrogen phosphate, peptone and 0.5 per cent agar. The indicator of preference was bromthymol blue. Readings were made daily for one week, and acid production in twenty-four hour broth cultures was determined by titration. The cultural technique has been described by Torrey and Buckell.

The organisms were cultured in one per cent gelatin in hormone broth for seven days at 37° C and were placed on ice daily long enough to permit setting of the gelatin. Indol, methyl red, and Voges Proskauer tests were made as for fecal organisms.

The growth was studied at 37° C in deep cultures, and in sealed hanging drop cultures on a warm stage microscope with which the organisms could be kept under continuous observation. As controls for the bacteriologic procedures and for comparative purposes the following groups of organisms were studied simultaneously: (1) three similar strains of higher bacteria from the respiratory tract, D, E, and F, (2) four strains of hemolytic streptococci, (3) one strain of *S. undans*, (4) three types of pneumococci, (5) fifteen strains of *S. fecalis* isolated from polluted water and human feces, and (6) one strain of *Actinomyces hominis* (American Type Culture Collection).

The pathogenicity of the genitourinary strains was tested in mice, guinea pigs, and rabbits by peritoneal intramuscular, and subcutaneous injections and by oral administration. The serologic relationship was studied by agglutination tests with immune sera for strains A, B and C tested against the genitourinary and respiratory strains.

#### BIOLOGIC CHARACTERISTICS

**Morphology**—In fluid cultures the organisms resembled long chained, short chained, and lancet shaped diplococci (Table I). A conspicuous feature was the parallel arrangement of the long chains (Plate II, Fig. 3). Variation in morphology gave rise to transverse constriction and to irregularity in the size and shape of the separate protoplasmic elements. True (dichotomous) branching was noted occasionally (Plate I). Branched or extremely long filaments were rarely seen in smears made directly from the patient, the predominating form in the secretions being that of a diplococcus with pointed distal ends. In stained films a thin walled sheath, prominent in hanging drop preparations was vaguely outlined (Plate II, Fig. 1). Pleomorphism was a constant characteristic of cultures. Young cultures were fairly uniform, cultures more than twenty-four hours old contained many bacilli, diplobacilli, short chains, long chains, and tangled masses. Globoid bodies appeared as the culture aged but were not seen to develop further. The filaments which appeared flattened with somewhat depressed centers, varied in width from 0.6 to 1.7 microns and in length from 2.2 to over 100 microns.

Growth on solid media gave a diplococci type of organism with pointed distal ends in pairs and short chains. Chains of six and eight elements generally possessed one terminal element of oval shape and of larger size than the other elements. The sheathed filaments were positive with the alkaline modification of the Gram method of staining. The organism did not take the acid fast stain. Often gram negative and gram positive elements appeared in

alternating sequences within the same filament Likewise, the large terminal body at times appeared gram positive when the rest of the filament appeared gram-negative

The organism is nonmotile, although in hanging drop preparations changes in the position of the filament occur from time to time, which may be ascribed to motility or to passive movement by diffusion currents The globoid and lancet shaped bodies appear to have an independent motion

TABLE I  
CULTURAL CHARACTERISTICS

MEDIA		MACROSCOPIC	MICROSCOPIC
Meat infusion agar	With blood	Colonies pin point, at first moist and semitransparent, later opaque and flattened, and green or rusty brown in color Nonhemolytic Strain C gives a small light halo Discoloration of surrounding medium	Lancet shaped diplococci in chains and pairs, frequently one terminal cell is larger than the other cells in the filament Gram positive in young cultures, becoming gram negative within forty eight hours
	Without blood	Similar to blood agar Colonies greyish white, pin point, becoming opaque with age, edges may be serrated and surface flattened Maximum diameter 1.0 mm	Similar to blood agar
0.5 per cent meat infusion agar		No surface growth, slight greyish growth below discrete with radiate outgrowths, 0.5 to 1.5 mm in diameter The greater the depth, the larger and more radiate the colony	Short and long chained diplococci Pleomorphism noticeable, but not so marked as in broth cultures
Meat infusion broth	P <sub>H</sub> 7.6	A web like structure with long interlacing fluffy filaments suspended from meniscus or adherent to wall of tube until disturbed Supernatant fluid clear after sedimentation Strain C shows less tendency to web formation and slightly clouds the supernatant fluid	Wavy bacilli, diplobacilli, and short chains in young cultures Flattened filaments of marked pleomorphism and length, and individual globoid bodies in old cultures Gram positive young cultures becoming gram negative after twenty four hours
	P <sub>H</sub> 6.8	Web formation less pronounced	Less marked pleomorphism Filaments short, especially if broth is shallow and contains fermentable carbohydrates
Sugar free infusion media		Negligible or slight growth	Usual pleomorphism
Potassium monohydrogen phosphate, peptone and dextrose		Negligible or slight growth	Usual pleomorphism

*Cultural Characteristics*—In broth the growth appeared consistently as creamy white tufts adherent to the walls of the culture tube by means of delicate, trailing filaments (Plate 3) In deep broth culture, particularly at P<sub>H</sub> 7.6, the growth formed as a delicate web-like structure which was suspended from the meniscus until disturbed, or until borne down by its own weight, leaving a clear supernatant fluid Small pieces of sterile cork enabled these web formations to be more securely festooned Although the supernatant liquid appeared clear to the naked eye, smears showed extremely small organisms in

chain formation. Transfers of the supernatant liquid to fresh broth gave rise to typical cultures. Strain C was less inclined to form webs and tended to cloud the supernatant broth which is consistent with its shorter chain morphology.

In semisolid agar no surface growth occurred but growth was greyish and semidiffuse below the surface and deep growth gave rise to discrete, radiate colonies. Facultative anaerobiosis occurred consistently.

On solid agar the colonies were plaque like and ranged in diameter from 0.1 to 10 mm. The typical colony had a depressed center and a concentric ridge which appeared between the center and the periphery. The margin was

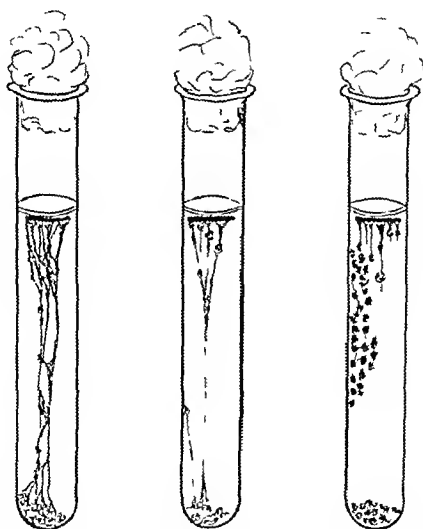


Plate III—Strains A, B, and C. Broth cultures in festooned growth from pieces of sterile cork.

seriated and noticeably fringe like when the surface of the medium was moist. Colonies which by chance grew between the solid agar layer and the sides of the glass tube or plate became scale like and in the presence of blood had a dark color.

On blood agar the colonies appeared greenish and nonhemolytic although Strain C produced a faint halo in the surrounding medium. The greenish color of the colonies was particularly pronounced if the blood was added to the melted agar at 60° C.

The genitourinary strains grew best at 37° C. No growth occurred at ice box temperature. Sluggish growth took place at 22° C. Strain C showing scarcely any growth. Death resulted at temperatures above 53° C. in ten



minutes. The optimum growth occurred at a hydrogen-ion concentration of  $P_H$  6.9 to 7.2. Strains A and B gave a slight growth at  $P_H$  6.1 to 6.3 and Strain C practically none.

In synthetic media, composed of potassium monohydrogen phosphate one per cent peptone and one per cent dextrose, no growth occurred within the first twenty-four hours with the genitourinary strains or with any control organisms except the fecal streptococci and *Actinomyces hominis*, which could be maintained in this medium for many generations. The organisms required the same selective media as the pneumococci and streptococci.

*Physiology*—Table II gives a summary of the cultural and physiologic characteristics of our strains as compared with those of known chain cocci. None of the organisms produced indol, reduced nitrates, or gave a positive Voges-Proskauer reaction. The methyl red test was negative except for the fecal streptococci. Two strains of hemolytic streptococci liquefied gelatin. Our strains did not liquefy gelatin and, like the streptococci, they were insoluble in bile. They fermented dextrin, galactose, glucose, levulose, maltose, and saccharose. They did not ferment arabinose, dulcitol, inulin, rhamnose, mannite, salicin, and xylose. Variable results were obtained with lactose for the genitourinary and with raffinose for the respiratory strains.

The type of fluffy web like growth in broth, the radiate colonies in 0.5 per cent agar, the plaque-like colonies with fringing of the periphery, and the filamentous morphology differentiate them from the streptococci and pneumococci. The agar colonies are smaller, less dry, and do not have the yellow color or the mold-like appearance of the old *Actinomyces hominis* colonies. The genitourinary strains differ from the pneumococci by not fermenting inulin and raffinose, and from the fecal streptococci by failing to ferment arabinose, mannite, and salicin. In general the growth of streptococci and pneumococci was more diffuse in fluid media. The growth of all strains except the fecal streptococci and *Actinomyces hominis* was erratic in synthetic media containing standard amounts of sodium and potassium phosphate. Our strains differ from the enterococcus of French and German literature inasmuch as they do not ferment mannite and have a thermal death point under 60° C.

The fecal streptococci differed from the other streptococci, pneumococci, and our group of higher bacteria by (1) profuse growth in potassium monohydrogen phosphate with one per cent dextrose, (2) acid range, (3) rapid production of acid in dextrose broth, (4) positive methyl red reaction, and (5) fermentation of arabinose and mannite.

*Bacteriologic and Serologic Grouping*—All attempts to show pathogenicity in mice, guinea pigs, and rabbits have failed, but the organism has been recovered from the heart's blood of mice twenty-four hours after intraperitoneal inoculation. Immune sera were produced in rabbits for the three genitourinary strains. Agglutination tests were made with the three sera, using as antigens strains A, B, and C isolated from the genitourinary tract, and in addition strains D, E, and F, isolated from the respiratory tract. Owing to the flocculent character of the growth, agglutination was difficult, but by using

TABLE II.  
COMPARISON WITH STREPTOCOCCI AND PNEUMOCOCCI

	GENTOURINARIA A B C	RESPIRATORY D E F	PNEUMOCOCCI	STREPTOCOCCI			ACTINOMYCES HOMINIS
				HEMOLYTIC	VERIDANS	FECALIS	
Morphology	Pleomorphic	Pleomorphic	No pleomorphism	No pleomorphism	No pleomorphism	No pleomorphism	Pleomorphic
Growth in broth	Fluffy, web like	Fluffy web like	Diffuse	Granular or dif- fuse	Granular or dif- fuse	Diffuse	Pellicle Discrete
Growth, semisolid agar	Radiate fringing of periphery	Radiate fringing of periphery	Diffuse	Granular or dif- fuse	Granular or dif- fuse	Diffuse	fluffy masses
Carbohydrate fermenta- tion	-	-	-	Variable	Variable	-	Radiate
Sabour	-	-	-	-	-	+	
Mannite	-	-	+	-	-	+	
Raffinose	-	-	+	-	-	-	
Inulin	-	-	-	-	-	+	
Arabinose	-	-	+	-	-	+	
Bio solubility	-	-	-	-	-	+	
Growth in potassium phos- phate	-	-	-	-	-	+	+
Methyl red	-	-	-	-	-	+	

the supernatant portion of rapidly-growing broth cultures, a uniform suspension was obtained. Serum dilutions ranged from 1:10 to 1:800. The approximate agglutination titer was 1:400.

Sera A and B agglutinated equally strains A and B but not strain C. Sera A and B agglutinated respiratory strain D, but more weakly than strains A and B, but did not agglutinate strains E and F. Serologically, genitourinary strains A and B were closely related to each other and were unlike strain C. Of the three respiratory strains, D showed some relation to A and B but none to C, while E and F were not related to A, B or C. Two serologic types were present in the genitourinary strains and at least one other in the respiratory strains.

TABLE III  
BACTERIOLOGIC AND SEROLOGIC GROUPING

STRAINS	AGGLUTINATION WITH IMMUNE SERA			CARBOHYDRATE FERMENTATION	
	A	B	C	LACTULOSE	LACTOSE
Genitourinary					
A	++	++	-	-	+
B	++	++	-	-	-
C	-	-	++	-	+
Respiratory					
D	+	+	-	-	+
E	-	-	-	+	+
F	-	-	-	+	+

The serologic relationship of these strains did not correspond with their groupings by cultural characteristics. By direct agglutination the six strains fell into three serologic groups, (1) A B D, (2) C, and (3) E F, as compared with the bacteriologic groups (1) A C D, (2) B, and (3) E F. Strain D, which bacteriologically was classed with A and C, serologically was related to A and B. Strain C was sharply defined from A and B by cultural characteristics.

*Identification*—A satisfactory identification of our organisms cannot be made owing to the prevailing confusion in regard to the classification of higher bacteria. They possess certain of the characteristics of the various groups which have been somewhat indiscriminately described as *Cladothrix*, *Streptothrix*, *Nocardia*, and *Aetnomyces*. The generic name *Cladothrix* is in disrepute and the terms *Nocardia* and *Streptothrix* are under critical consideration. Our organisms are quite unlike the classical *Aetnomyces* and *Streptothrix*, most of which give hardy, tenacious, and at times mold-like growth on solid media. They have certain points in common with the classical *C. dichotoma* of Collin, though definitely at variance in others. Morphologically and culturally, particularly in respect to the plaque-like colonies, they seem consistent with Kligler's description of *Cladothrix placoides* isolated from dental caries.

The group within itself seems diversified, the individual strains varying widely in cultural and serologic characteristics. Further study of a larger number of strains may give additional information in regard to their generic relationship and to their association with pathologic processes.

## DIAGNOSIS

The methods of differentiation between gonorrheal and nongonorrheal urethritis and vaginitis are time consuming. Bacteriologic diagnosis of gonococcus infection depends upon the identification of the gonococcus by morphology and arrangement in stained films of genitourinary secretions and upon positive cultures. The demonstration in films of the typical diplococcus of Neisser and the absence from culture of organisms which could be mistaken for it seem satisfactory evidence of gonococcus infection. Consistent negative results with carefully repeated smear and cultural examinations over a period of four to six months especially when another organism predominates in the cultures would seem sufficient to warrant a diagnosis of nongonorrheal infection. However chronic gonorrhea during latent periods may give a picture of nonspecific infection with other organisms.

Baker claims that 15 to 20 per cent of genitourinary cases in private practice are of nonvenereal origin. Our observations suggest that about the same per cent may be true for vaginitis and cervicitis in children.

The mucous surfaces afford a favorable habitat for the higher bacteria. Several instances of pulmonary and general infection of *Streptothrix* or *Nocardia* have been reported. Khigler studied fifty eight strains of *Cladothrix placoides* associated with dental caries. Bacteria similar to our strains have been found in the respiratory tract under normal and pathologic conditions.

The incidence in the normal mucosa of the genitourinary tract is unknown, since all organisms reported in the literature have been obtained from patients with pathologic conditions. Paul and Williams recovered *Nocardia* from several cases of stillbirths with invasion of the placenta. Celler and Thalheimer described three strains of streptobacilli repeatedly isolated from catheterized urine and associated respectively with chronic prostatitis, tumor of kidney, and edema bulbosum vesicalis. These organisms similar in many respects to our strains, was nonpathogenic for rabbits and guinea pigs, but was recovered regularly from the urine forty eight hours after intravenous inoculations.

In order to obtain an approximate idea of the prevalence of these organisms in nongonorrheal infections twelve patients with genitourinary inflammation without the presence of the gonococcus were examined as to causative organisms. Two gave our group of higher bacteria in pure culture, one hemolytic streptococcus, three gram positive bacilli similar to *Doderleins bacillus*, and six a mixture of bacteria, diphtheroid bacilli predominating.

The association of our organisms with chronic inflammation of the genitourinary tract raises the question of their pathogenicity. Although they appear incidental or secondary invaders rather than primary pathogens chronic irritation seems to favor their existence. Two of our strains were obtained from patients with a gonorrheal history and one from a patient with urethritis following a probable streptococcal infection. Against pathogenicity are their absence in acute inflammation, the presence of similar strains in the respiratory tract though usually from abnormal mucosa and then nonpathogenic action in laboratory animals. In favor of their pathogenicity is their presence in practically pure culture in chronic inflammation long after the disappearance of the primary organisms.

## SUMMARY

1 Three strains of higher bacteria possessing certain characteristics of the *Cladothrix* and *Streptothrix* of the early bacteriologists have been isolated from the genitourinary tract of patients with chronic urethritis. One of the three strains is culturally and serologically distinct from the other two.

2 Owing to the confusion in the classification of the higher bacteria, these organisms cannot be accurately placed in the present scheme of classification. Further study of many similar strains will be required before there can be a final identification of this group. The cultural and physiologic characteristics of three genitourinary and three respiratory strains are compared with various types of pneumococci and streptococci, in order to record their characteristics.

3 Their relationship to chronic inflammation of the genitourinary tract may be secondary and incidental, although there is some evidence to indicate that they prolong the chronic process.

4 In spite of their pleomorphic character, they may be mistaken in smear and isolation culture for streptococci. They are differentiated morphologically by the thin wall sheath.

5 Bacteriologic and serologic characteristics indicate that the group is composed of diverse strains. The bacteriologic and serologic groupings do not correspond. A serologic relationship was shown between a strain isolated from the respiratory tract and two from the genitourinary tract.

6 In comparative studies it was found that fecal streptococci are differentiated from these organisms and from other types of streptococci by the fermentation of arabinose and mannite, rapidity of growth in dextrose potassium monohydrogen phosphate with peptone, rapidity of acid production in dextrose broth, and a positive methyl-red test.

We are indebted to Dr. John Cunningham and Dr. Roger C. Graves of Boston for the use of clinical material from two patients, and to Mr. John A. Seaveins for the method of producing the colored photographic plates.

## PHOTOMICROGRAPHIC TECHNIC

The photomicrographs accompanying this paper were made by John A. Seaveins of Boston. They are unusual in that they secure two color separation with a single exposure. This method eliminates many difficulties and secures sufficiently good color value for classroom purposes.

The technic, which is quite simple, is as follows:

The original exposure is made with standard photomicrographic apparatus, using a panchromatic plate and a red filter. The negative thus obtained is printed by contact or enlargement on a lantern plate. When this latter plate is dried, it is used as a negative to print a second lantern slide and the pair so obtained bleached in a special bleaching bath and when thoroughly washed, stained with the regular stain used for the original micro slides. When dry, these two plates are bound with their film surfaces in contact, which, as they were printed one from the other, insures accurate registration without complicated technic. The original negative is preserved with care and

black and white lantern slides are made as usual. In making halftone printing plates, a pair of lantern plates are made but not bleached and are either used direct or as negatives for two glossy prints from which the halftone exposures are made.

This process, while not separating all the colors as well as the method of two separations from the original using selected green and red filters, makes slides which are, in general, satisfactory and avoids the extreme care necessary when two separations are made successively from the same subject, since it is difficult to avoid movement of the apparatus between exposures thereby causing lack of registration in the finished print. When using the two exposure, two filter method, the printing must be done in a projection apparatus in order that the two film sides of the compound lantern slide may be in contact and be registered. This two exposure technique involves special apparatus and its accomplishment is extremely difficult since even slight differences in the thickness of the glass in the original pair of negatives will alter the registration. There are certain modifications in the filters which may be used for subjects having unusual color value and such filters are not difficult to obtain.

#### REFERENCES

- Baker, T. J. A. M. A. 85 1606 19-5  
 Celler H. L. and Thalheimer W. J. Infect Dis 21 503 1917  
 Kilgier I. J. J. Allied Dental Soc 10 282 1915  
 Park W. H., and Williams A. W. Pathogenic Microorganisms, 1920 page 229  
 Scudder S. A. J. Urol 14 429 1925  
 Torrey J. C., and Buckell G. T. J. Infect Dis 31 125 1922

## THE SPECIFIC GRAVITY OF THE BLOOD ITS CLINICAL SIGNIFICANCE\*

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THE clinical significance of the specific gravity of the blood has not received the recognition it deserves. In the falling drop method for determining the specific gravity of fluids Bibb and Hamilton<sup>1</sup> have removed practically all the difficulties formerly associated with that determination. Only one drop of blood, obtained in the same manner as for blood counting or hemoglobin calculation, is required.

One can readily conceive that a knowledge of blood density in disease should be of equal if not of greater importance as the routine determination of the specific gravity of the urine. The following investigation is therefore, reported with the view of establishing the value of the routine determination of the specific gravity of the blood in disease.

*Normal Densities of Human Blood*—Bamberger cited by Lyonnet placed normal blood densities at an average of 10575 for males 1053 for females

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Schmaltz<sup>3</sup> found them higher with males at 1059, females 1055, with afternoon densities lower by about 0.002. Leake, Kohl, and Stebbins,<sup>4</sup> using the falling drop method of Barbour and Hamilton, found the average densities for males to be 10565, for females 10533, with a maximum diurnal variation in the blood density of males being 0.0033, of females 0.0027. Polowe, also using the falling drop method, made fifty-two observations on ten normal persons. He placed the average blood densities for males at 1057, females 1054, with the afternoon density drop averaging about 0.002. With these findings in mind normal blood densities for humans may reasonably be said to be between 1050 and 1060, that they are higher in males and higher in the morning than in the afternoon.

TABLE I  
MISCELLANEOUS

OBSERVATION	BLOOD DENSITY	DIAGNOSIS
46	1032	Syphilitic stricture of rectum
62	1032	Severe Hg poisoning (died)
48	1033	Perniciou anemia
30	1034	Tb pleurisy with effusion
51	1034	Portal cirrhosis Ascites
57	1035	Pancreatic Cyst
47	1037	Internal hemorrhoids
59	1037	Achylia gastrica with enteritis
102	1039	Enteritis of unknown origin
112	1041	Chronic myelogenous leucemia
74	1043	Perniciou anemia
4	1047	Rheumatic pneumonia
34	1047	Senility (85 yr old)
67	1047	Perforated peptic ulcer
21	1051	Pneumoperitoneum
32	1051	Hypertrophie prostate, benign
73	1051	Osteosarcoma of mandible with metastasis to lung
104	1052	Duodenal ulcer
3	1054	Hemiplegia
23	1054	Head injury
83	1054	Pneumothorax with lung abscess
90	1055	Breast adenoma, benign
101	1055	Submucillary adenitis
18	1056	Cerebr. Apoplexy
61	1056	Perniciou vomiting of pregnancy
84	1056	Obstructive jaundice
97	1057	Ulcer at pyloric end of stomach
105	1057	Syphilis
85	1058	Compound fracture of elbow
77	1061	Bronchial asthma

*Clinical Blood Density*—Copeman<sup>1</sup> cites Christison on the density of blood serum in early nephritis, when it is considerably lowered and may be as low as 1019, the normal specific gravity of the blood serum being about 1028. Schmaltz<sup>3</sup> concludes his extensive study of blood density by stating that blood density varies within narrow limits in health and varies considerably under pathologic conditions. Lyonnet<sup>2</sup> cites Lloyd Jones' findings in nephritis, the blood density in parenchymatous nephritis being 1034, in interstitial nephritis 1062 to 1042. A differential diagnosis between cardiac and nephritic is suggested in that the blood density in cardiac is about 1059, in nephritis 1052. Bender and Polowe,<sup>7</sup> in a series of seventeen spinal anesthetics,

have found that with the fall in blood tension that follows the administration of the spinal anesthetic there is a concomitant fall in blood density. At the very beginning of the anesthesia there is a transitory increase in blood density. This is followed very soon by a small but definite fall in blood density. As the patient begins to recover from the anesthesia the rise in blood tension is followed by an increase in blood density.

For the purposes of this study the blood densities of 85 hospital and clinic patients were arranged in ascending order. Twenty four per cent of the blood densities were below 1040, 42 per cent below 1050. In the miscellaneous group (Table I) from which were omitted the cases of proved

TABLE II  
CARCINOMAS

OBSERVATION	BLOOD DENSITY	LOCATION
88	1032	Gastrum
28	103	Urinary bladder
8	1037	Pancreas
69	1039	Urinary bladder
58	1041	Peritoneum
115	104	Rectum
81	1044	Colon
93	1044	Pancreas
116	1047	Peritoneum
114	1048	Submaxillary glands
100	10 1	Bladder
6	10 3	Lung
111	10 3	Esophagus
96	10 4	Cervix
87	10	Cervix
71	10	Esophagus
9	10	Alimentary
86	10 ,	Tonsil
106	10 ,	Breast
107	10 ,6	Breast
91	10 ,7	Breast
92	10 ,8	Breast
94	10 ,8	Tongue

TABLE III  
CIRCULATORY RENAL DISTURBANCES

OBSERVATION	BLOOD DENSITY	DIAGNOSIS
29	10.9	Congenital atrophy of right kidney
52	1029	Chronic nephritis with death
51	1034	Pancarditis death
5	1037	Acute arthritis with acute nephritis
66	1039	Pancarditis death
4	1041	Cardionephritis
14	1047	Polycystic kidney
6	10 1	Auricular fibrillation
66	10 1	Myocarditis death
24	105	Chronic nephritis
78	1053	Low reserve kidney eclampsia
53	10.5	Myocarditis hypophoria death
82	1050	Aortic aneurysm
79	1056	Cardionephritis
98	1057	Subacute nephritis
0	1058	Myocarditis with heart block chronic nephritis
10	10 9	Acute fever with auricular fibrillation



carcinoma (Table II), of circulatory-renal disturbances (Table III), and of diabetes mellitus (Table IV), 30 per cent of the blood densities were below 1040, 50 per cent below 1050. Fourteen cases exhibiting fluid collections in tissues or serous cavities are listed in Table V.

*Carcinoma* (23 cases) —Carcinoma has long been a factor in the production of secondary anemia. The cases here reported were nearly all ad-

TABLE IV  
DIABETES MELLITUS

OBSERVATION	BLOOD DENSITY	URINE DENSITY	GLYCOSURIA	INSULIN ADMINISTERED
17	1032	1020	yes	no
20	1039	1027	yes	yes
21	1010	1023	no	no
22	1047	1016	yes	no
16	1049	1020	yes	no
14	1051	1020	yes	yes
18	1053	1022	no	no
9	1053	1029	no	no
19	1053	1020	yes	no
8	1054	1020	no	yes
11	1054	1015	no	?
12	1054	1017	yes	yes
15	1055	1022	yes	no
13	1056	1025	yes	yes
10	1057	1037	yes	yes

TABLE V  
FLUID COLLECTIONS

OBSERVATION	BLOOD DENSITY	LOCATION OF FLUID	DIAGNOSIS
51	1034	Fluid in abdomen	Portal cirrhosis
57	1035	Pancreatic cyst	Traumatic
9	1038	Pleural effusion	Tuberculosis
76	1039	Fluid in abdomen	Pancreatitis
54	1041	Fluid in abdomen	Cardionephritis
58	1041	Fluid in abdomen	Carcinoma peritoneum
93	1044	Fluid in abdomen	Carcinoma pancreas
116	1047	Fluid in abdomen	Carcinoma peritoneum
45	1050	Edema extremities	Tricuspid insufficiency
56	1052	Edema extremities	Cardiac
65	1053	Pleural effusion	Carcinoma lung
79	1056	Edema extremities	Cardionephritis
105	1057	Edema extremities	Syphilis, late with diabetes mellitus
103	1059	Edema extremities	Acute rheumatic fever with auricular fibrillation and cardiac decompensation

vanced cases proved by biopsy or necropsy. Ten, or 43 per cent, had blood densities below 1050. Of these seven (70 per cent) obtained in carcinoma of the gastrointestinal tract or associated structures, the carcinoma of the stomach exhibiting the most marked grade of anemia. These findings are in keeping with those of Mayo<sup>9, 10</sup> who found that carcinoma of the proximal half of the colon tended to produce anemia more than any other part of the body, except carcinoma of the median portion of the stomach. The statistical work of Alvarez, Judd, MacCarthy, and Zimmermann<sup>11</sup> is in cor-

roboration with Mayo in respect to anemias produced by carcinoma of the cecum and ascending colon. At Rochester the belief is that the main factor in the production of anemia in carcinoma of the gastrointestinal tract is the presence of large ulcerated areas from which there is a constant oozing of blood and through which bacteria gain easy access to the body.

More recently Alvarez and MacCarty<sup>1</sup> have shown that 92 per cent of gastric ulcers of a size less than a silver quarter are benign; those over a silver dollar in size are almost certainly malignant. One can readily see that there likewise may be a difference in the degree of anemia directly proportional to the size of the ulcer. While secondary anemias occur in benign as well as malignant gastric ulcer, the fact of anemia would incline one toward a diagnosis of benign ulcer. In other words, given a case in which the clinical history points definitely toward the presence of a gastric ulcer, the finding of a normal red blood count, hemoglobin or blood density (or if these are only moderately reduced) would favor the diagnosis of benign ulcer.

Carcinoma of the urinary bladder seems also to produce a severe anemia; the blood density in two of the three cases reported being below 1040. Here again, perhaps the ulcerated area offers a portal of escape of blood and of entrance to bacteria.

Attention is invited to the fact that in this series, the more severe anemias as evidenced by blood densities below 1000 occurred in carcinoma of hidden or not readily accessible areas (gastrointestinal tract, peritoneum, pancreas, urinary bladder). Carcinoma of other parts of the body did not seem to alter the normal blood density, even though most of the cases were quite advanced. This appeared to be particularly true of carcinoma of the breast and of the cervix.

All these facts should be taken into consideration when evaluating the presence of secondary anemia, especially where the symptoms point toward involvement of the gastrointestinal tract.

*Circulatory Renal Disturbances* (17 cases).—Forty-one per cent of this group exhibited blood densities below 1000. Seventy-one per cent of these were in cases having severe renal involvement and are in accord with the findings of Lloyd Jones, Christison and Barbour<sup>2</sup> and Dawson. Barbour and Dawson state that the low colloidal pressure is reflected by a lowered serum specific gravity in cases of renal involvement.

*Fluid Collections* (14 cases).—Since specific gravity of the blood is closely bound up with water balance, it was considered profitable to study the blood density in cases exhibiting fluid collections in tissues and in the serous cavities. A very striking finding here is the fact that eight cases (57 per cent of the total) had blood densities below 1000 and that all of these (100 per cent) presented free fluid in serous cavities. In only one case, pleural effusion in a carcinoma of the lung, was a blood density of over 1050 concomitant with free fluid in a serous cavity. In four cases the free fluids were exudates, three transudates, and two were not studied from this angle.

These findings would appear to predicate extraordinary changes in blood density before free fluid finds its way into serous cavities

*Diabetes Mellitus* (15 cases) —Thirty-three per cent of the cases here presented exhibited blood densities below 1050 In 80 per cent of these glycosuria was present Glycosuria was present in only 60 per cent of the cases having a blood density greater than 1050 A further study of this phase of blood densimetry might bring some important light to bear on the relation of blood density to the renal threshold of sugar and to the dosage of insulin

*Comment* —Definite conclusions cannot be drawn from so small a series of observations However, it appears likely that blood density determinations should be helpful in diagnosing such conditions as carcinoma of the gastrointestinal tract, cardiorenal disturbances and water imbalances involving fluid collections in serous cavities

It may be stated in closing that routine determinations of blood density in disease should be encouraged Such blood density determination most certainly rounds out the laboratory picture and in some clinical entities may be a definite guide toward accurate diagnosis

#### REFERENCES

- 1 Barbour H G, and Hamilton, W F The Filling Drop Method for Determining Specific Gravity of Fluids, *J Biol Chem* 69 625, Aug, 1926
- 2 Lvonnet, Le Dr B De La Densité Du Sang Sa Détermination Clinique, Ses Variations Physiologiques et Pathologiques, Paris, J B Baillière et Fils, 1892
- 3 Schmaltz, von Dr Richard Die Untersuchung des Spezifischen Gewichtes des Munchischen Blutes, *Deutsche Arch f klin Med* 47 145, 1891
- 4 Leake, C D, Kohl, M, and Stebbins, G Diurnal Variations in the Blood Specific Gravity and Erythrocyte Count in Healthy Human Adults, *Am J Physiol* 81 493, July, 1927
- 5 Polowe, David The Blood Urine Density Index, *J LAB AND CLIN MED* 12 11 1100 1107, Aug, 1927
- 6 Copenan, S Monkton Report on the Specific Gravity of the Blood in Disease, *Brit M J* 1 161, Jan 24, 1891
- 7 Bender, Theodore, and Polowe, David Blood Density and Blood Tension During Spinal Anesthesia, unpublished data, from the Gynecological Service of the Barnert Memorial Hospital
- 8 Barbour, H G, and Hamilton, W F *Am J Physiol* 69 654, 1924
- 9 Mayo, W J Some of the Disputed Problems Associated with Surgery of the Large Intestine, *Am J M Sc* 145 159, 1913
- 10 Mayo W J Pernicious Anemia with Special Reference to the Spleen and the Large Intestine *Ann Surg* 74 355 Sept, 1921
- 11 Alvarez, W C Judd, E S, MacCarty, W C, and Zimmerman, A R The Varying Degrees of Anemia Produced by Carcinoma in Different Parts of the Colon, *Arch Surg* 15 402, Sept, 1927
- 12 Alvarez, W C, and MacCarty, W C Sizes of Resected Gastric Ulcers and Gastric Carcinomas, *J A M A* 91 No 4, 226, July 28, 1928

## INFECTIOUS MONONUCLEOSIS\*

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THE temporary increase of the large mononucleated cells of the blood (symptomatic mononucleosis) is observed in various diseases as in malaria, varicella, German measles, kala azar, recurrent fever, amebic dysentery, streptococcus septicemia after angina Vincent's angina peritonitis diphtheria, typhoid fever, typhus, acute pulmonary tuberculosis lymphogranulomatosis, etc., and is well known (Meiklen and Wolf Moscovitz) Schilling also reports the occurrence of a monocytic phase between the leucocytic and eosinophilic phase in suppurative diseases. In more recent years cases on an apparently infectious basis were reported in which the main symptoms consisted in more or less generalized swelling of the superficial lymph nodes and an increase of the monocytes together with an absolute leucocytosis and absolute and relative lymphocytosis. As the nature of this peculiar blood reaction is still unknown and the number of reports published on this subject rather small, a brief discussion of the clinical symptoms and pathologic findings thus far known may be of interest especially as several cases of this type of disease came to observation in recent months in Mercy Hospital.

### HISTORICAL DATA

In 1907 Turk and later Marchand Deussing Jagie and Schuffner Pribram and Stern, Haloi Tody and Daniel described cases of a peculiar type of angina in which a mononucleosis and a swelling of the lymph nodes were the main characteristics. Schultz published in 1922 a series of such cases and called the disease "monocytic angina" or "lymphoid cell angina" which is probably identical with Pfeiffer's Druesenfieber (1889). The Americans Sprunt and Evans and Bloedorn and Houghton observed similar blood findings in patients with inflammatory processes of other organs and named the disease infectious lymphomonocytosis or 'acute benign lymphoblastosis' respectively. Similar observations were made by others and diagnosed as "acute benign lymphatic leucemias" or infections with lymphatic blood reaction.

### SYMPTOMATOLOGY OF MONOCYTIC ANGINA

The onset is rather sudden starting with headache malaise chilly feeling or sometimes real chill high fever of intermittent character sore throat, which may also appear somewhat later and dysphagia. There may be a generalized tender swelling of the superficial lymph nodes especially the sub

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mandibular and cervical ones, which may vary in size from that of a bean to that of a date, rarely larger. The lymph-node enlargement may sometimes precede the sore throat. An edematous swelling of the face is also occasionally seen. The patient appears to be seriously ill.

The physical examination gives following findings:

*Mouth* The tonsils are enlarged and covered completely or partly by a brownish to blackish coat which is usually restricted to these organs but which may sometimes extend to the uvula. In later stages dirty looking, suppurating, eroded surfaces are seen. Then tonsillitis is either of a lacunary or membranous type. Breath is fetid.

*Neck* Swelling and tenderness of the lymph nodes.

*Thorax* Normal findings.

*Abdomen* Enlargement of the liver and spleen are usually observed. The spleen is firm but not tender. The swelling of the spleen may persist for several months after clinical recovery.

Jaundice is in general absent but occurs occasionally (Schmidheiny). Hemorrhagic diathesis of a generalized character is not present, scanty, scattered hemorrhages may exist (Downey and McKinley, Schmidheiny, Baader, Schwenkenbecher).

#### LABORATORY FINDINGS

The bacteriologic examination of the throat by smear and culture shows the presence of streptococci and staphylococci and the frequent occurrence of a fusospirochlosis. Diphtheria bacilli are always absent. The urine contains in general a small amount of albumin. Blood. A granulocytic leucocytosis sometimes exists for a short period in the beginning. It is followed by an absolute leucocytosis (usually 20,000-30,000, occasionally higher, up to 50,000 and 80,000) and an absolute and relative lymphocytosis and monocytosis (usually 6 to 9 per cent, but going up to 76 per cent), which is especially marked at the climax of the disease. This condition may prevail to a minor degree even during the period of convalescence, or the number of leucocytes may drop below the normal level and a leucopenia with lymphocytosis may be present (Weiss, Richards). The red blood picture is normal, and the number of platelets is either normal or increased.

Monocytes are large cells with a deeply basophilic cytoplasm, which is often vacuolated, and large, indented or lobulated, sometimes eccentrically placed nuclei with a heavy chromatin network. Nucleoli are rarely present. The oxydase reaction is usually negative, but sometimes fine gray purple granules are observed instead of the coarse blue ones in granulocytes, or even a definitely positive result may be seen. Most of the authors agree that no conclusions upon the genesis of the monocytes can be drawn from the result of this test. The mononucleated cells of the blood are represented by the small and large lymphocytes, plasma-cells, lymphoblasts, myeloblasts and monocytes. The exact origin and characteristics of the monocytes is still an unsettled question. Three different tissues are named by workers on this subject as the possible sources of these cells which appear under various names in the literature (transitional cells [Ehrlich, Tink, Rieder], polyblasts [Maximow], leuco

cytic cells [Mallory], adventitia cells [Marchand], lymphoid cells [Pappenheim]) The three sources from which monocytes may originate are

- 1 The bone marrow (Naegeli)
- 2 The reticulo endothelial cells (Schullug, Kaznelson, Mallory, McJunkin)
- 3 The lymphoid tissue (Arneth Richards)

The monocytes of different origin differ from each other morphologically and functionally according to Moseyitz McJunkin divides the monocytes originating from endothelial cells into two groups, those coming from the endothelium of blood vessels (hemendothelocytes) and those from the reticulum of lymph nodes (lymphendothelocytes) The lymphendothelocytes show, according to this author after supravital staining with neutral red a red rosette like formation in the cytoplasm while the hemendothelocytes have a granular hyaline appearance Arneth asserts that the monocytes represent advanced developmental stages of large lymphocytes while Richards believes that they are lymphoid cells of an immature type The situation becomes still more complicated as there seem to exist transitions between lymphocytes, plasma cells and monocytes (Schultz) Naegeli, however, claims that plasma cells are either of lymphatic or myeloid origin while Schultz and Arneth consider them as exclusively lymphatic cells on account of their negative oxydase reaction In consideration of the different nature of the mononucleated cells of the blood Schultz classifies the anginas characterized by a mononucleosis into three types according to the type of mononucleated cell predominant at the climax of the disease

- 1 Plasmacellular lymphatic lymphoid cell angina
- 2 Monocytic angina
- 3 Myeloblastic angina

He notes that the first two mentioned types are the most important and frequent ones and believes that the plasmacellular type is probably only a morphologic variation of the monocytic type He is not sure about the actual existence of the third mentioned type which is clinically accompanied by a cutaneous purpura But in the cases published by Hertz Krumbhaar Halen Selwenkenbecher the majority of the monocytes showed a definitely positive oxydase reaction and Krumbhaar and Hertz designate these cells as myeloblasts

Experimentally a temporary monocytosis can be produced by the repeated intraperitoneal injections of India ink, collargol, lipoids proteins (Bungeler) trypan blue sudan III and other colloids and suspensions (red gold lamp black) (Witts, Simpson McJunkin and others) McJunkin effected a monocytosis by injections of tuberculous material into guinea pigs, also in injections with smegma bacilli were successful Sabin and Doan obtained similar results by injections of the phosphatid fraction of tubercle bacilli The monocytes increased after repeated injections of these substances from the normal figure of 3 to 5 per cent to 10 to 15 per cent Witts finally reported a considerable increase of the monocytes after a single injection of a vaccine of bacterium monocyto genes

# AN EXPERIMENTAL STUDY OF THE VALUE OF MERCUROCHROME-220 SOLUBLE AS AN ANTISEPTIC AGENT\*

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## INTRODUCTION

IN ORDER for any antiseptic to be considered effective in surgical usage, it must kill pathogenic bacteria under the conditions existing in the tissues. It is equally important that the antiseptic accomplish this object without serious damage to the body as a whole or to its various organs.

The purpose of this communication is to record the results of experiments in which these two criteria have been applied to mercurochrome 220 soluble†. It is necessary therefore first to determine the tolerance of the tissues to mercurochrome and then to observe the effectiveness of a tolerated concentration of the antiseptic in the treatment of local infections.

The first of these objects has been accomplished by means of an original and unique technique for testing the absolute tolerance of tissues to any substance, first suggested by Dr Barney Brooks, a technique which appears to reduce sources of error to a point as near the minimum as is humanly possible.

The determination of the tolerance of the tissues to mercurochrome involves two factors—the concentration used, and the time the tissues are exposed to the solution of the antiseptic.

## EXPERIMENTAL

*Tolerance of the Tissues to Mercurochrome*—Since the object was to determine the maximum concentration of mercurochrome and the time of exposure to this solution which the tissues could tolerate, it seemed necessary to find a means by which the tissues of a given part, an extremity, for instance, could be exposed to this when mercurochrome is injected intravascularly. If the mercurochrome is diluted by the volume of the circulating blood it is reasonable to assume that the concentration received by any given organ will vary with the richness of its blood supply. For experimental purposes intravenous injection of the drug has a further disadvantage in that its effects upon various organs are fused in a manner difficult to analyze. In order to obtain a true tissue tolerance it seemed best to isolate and empty temporarily the blood vessels of a part before filling them with mercurochrome.

We are indebted to Dr Barney Brooks for suggesting the original technique which accomplishes this. It permits the retention of a known concentra-

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†Hynson, Westcott and Dunning.

tion of mercurochrome in the tissues for a definite time. The routine procedure employed in all experiments is outlined below.

#### METHOD

One of the hind limbs of a dog was shaved carefully from the level of the groin down. A compression bandage of thin rubber like that used as a dental dam was applied to the extremity from below upward, beginning at the claws and extending to a level about 2 inches above the knee joint. A rubber tube was placed around the thigh to act later on as a tourniquet. In order to keep it from slipping down when later tightened, it was threaded under a stout cord, which snugly encircled the dog's body just above the iliac crests. With this special tourniquet in place but not tightened, the skin of the groin was sterilized in the usual manner with iodine and the animal anesthetized with ether. The femoral artery was exposed and lifted up by means of an encircling traction thread of silk. The tourniquet above was tightened until the pressure obliterated all pulsation in the artery. The bandage below was then removed, and a measured amount of mercurochrome at a given concentration and at body temperature was injected into the vessel until both it and the veins were filled under slight pressure.\* By employing this method the mercurochrome may be retained locally as long as desired before sweeping it into the general circulation by removal of the tourniquet. Rigid asepsis was maintained throughout all the experiments.

Before beginning the actual tests of tolerance the completeness with which the drug infiltrated the living tissues was determined. It was found that a few moments after the injection of 0.5 per cent mercurochrome solution pink splotches appeared in the skin which indicated the filling of the most superficial vessels. As the injection progressed the whole skin became uniformly stained. The knee joint of the animal was opened five minutes after the injection and both the joint fluid and the articular surfaces were found to be stained pink which indicated a complete infiltration of all the tissues of the extremity by the dye.

From the foregoing description we feel that the reasons why we adopted this method of testing tissue tolerance as an extremely fair and accurate one are evident. It is simple and safe in application, avoids the unnecessary sacrifice of animals and yields end points easy to interpret. Unlike general intravenous injection tests, it avoids the confusing pictures which may occur from the simultaneous effects of any drug upon several organs or from its rapidly changing concentration due to excretion. Finally it possesses one feature which Claude Bernard always emphasized to his pupils as being very valuable. It permits easy simultaneous control experiments to be run upon the same animal.

The following is presented as an experiment which typifies the technic employed in all the determinations of the tolerance of the tissues to mercurochrome.

\*The amount injected would vary in proportion to the size of the dog, but in each case the injection was continued gently until the artery would remain dilated with the solution and the pressure within it would cause a slight backflow into the syringe.



*Experiment*—A dog was anesthetized with ether, and a roller bandage of rubber dam was applied with slight pressure from the toes up to the inguinal region on the right side. The femoral artery was dissected free and a fine hypodermic needle inserted into it. A tourniquet of rubber tubing was applied around the thigh just above the incision and the pressure bandage removed. Ten cc of 0.5 per cent mercurochrome solution warmed to body temperature were injected very slowly. There was an instantaneous appearance of the characteristic pink coloration in the skin, slightly mottled in distribution at first but soon evenly distributed throughout the skin surface and deepening in intensity.

Fifteen minutes after the injection the circulation was reestablished by removal of the tourniquet and the wound was then closed.

*Result*—Twenty-four hours later the leg was somewhat swollen. Forty-eight hours after injection the mercurochrome was still slightly visible, and large dark sloughing areas were found on surfaces of both the thigh and the leg, but no generalized gangrene was present. The animal died the same day.

The results of 20 experiments, like the one described above, are summarized in Table I and Table II.

TABLE I

PRODUCTION OF GROSS PATHOLOGIC LESIONS BY HIGH CONCENTRATIONS OF MERCUROCHROME 220 SOLUBLE

TREATMENT		NUMBER OF ANIMALS TESTED	ANIMALS SHOWING GROSS PATHOLOGIC LESIONS (GROUP I)
CONCENTRATION OF MERCUROCHROME PER CENT	TIME OF EXPOSURE IN MINUTES		
0.500	15	3	3
0.500	5	2	2
0.350	15	1	1
0.350	5	1	1
0.130	15	1	1
0.065	15	2	2

TABLE II

TOLERANCE OF TISSUES TO MERCUROCHROME

TREATMENT		NUMBER OF ANIMALS TESTED	ANIMALS SHOWING GROSS PATHOLOGIC LESIONS (GROUP I)	ANIMALS SHOWING FUNCTIONAL EFFECTS ONLY (GROUP II)	ANIMALS UNAFFECTED (GROUP III)
CONCENTRATION OF MERCUROCHROME PER CENT	TIME OF EXPOSURE IN MINUTES				
0.014	5	1	0	1	0
0.0065	5	2	0	2	0
0.0033	10	2	2	0	0
0.0033	7	2	0	0	2
0.0033	5	3	0	1	2

From the results of the experiments on the tolerance of tissues to mercurochrome, it is apparent that the effects produced in the tissues by the different concentrations and times of exposure may be divided into three groups. Group I includes all gross pathologic lesions involving loss of tissue, such as localized or extensive gangrene. Group II includes mild effects functional in nature, involving no loss of tissue, but inconsequential changes, such as

slight edema, transient soreness, tenderness or lumping. These effects were interpreted as indicating irritation caused by the weaker concentrations. Group III includes animals which showed no effects following injection.

Certain ones of the first group with gangrene died and certain others were sacrificed to prevent unnecessary suffering. It was not thought important to express this in the tables since we are here primarily concerned with local tissue effects.

From Tables I and II it may also be seen that the effects produced are the direct result of the concentration used. At all concentrations above and including 0.065 per cent gangrene resulted from the injections. At 0.014 per cent, functional effects were produced while a percentage of 0.0033 was tolerated perfectly in the cases of four out of five animals when it was retained for periods of five minutes and in both animals tested at seven minutes.

As to the cause of the lesions in Group I of the first table the occurrence of gangrene might be thought to be caused by thrombosis due to intravascular clotting the predisposing factors being the manipulation of the artery for injection purposes and the conceivable injuries of applying the bandage and tourniquet. Evidence is available that the technique is not responsible for the production of these lesions. In the first place no injury resulted in experiments with control legs in which normal saline was substituted for the mercurochrome solutions. In the second place at autopsy thrombosis appeared in only three dogs in the entire series in which gangrene resulted after mercurochrome injection. In these three the thrombosis occurred after the appearance of gangrene. The first case showed thrombi in all vessels three days after injection, the second a thrombus occluding the right femoral vein ten days after mercurochrome and the third same findings in both the femoral artery and vein nine days after injection. Thus from both the standpoint of negative control experiments and the rarity and late occurrence of thrombosis it is evident that gangrene results from a diffuse effect of mercurochrome upon the tissue cells and not from primary thrombosis due to vessel injury.

In three of the experiments isotonic salt solution was used as a diluent for the mercurochrome in place of the distilled water usually employed. The purpose of this was to see whether an isotonic solution would be less irritating as a vehicle for mercurochrome than one like distilled water which is hypotonic. No difference in effect was observed.

The element of time in influencing the results is important. Solutions retained fifteen or even ten minutes before release of the tourniquet seem to permeate the tissues more thoroughly than those retained for five minutes. It may be assumed that the longer the mercurochrome remains within the artery at its full concentration the more tissue damage is accomplished. Thus concentrations of mercurochrome at 0.0033 per cent caused definite pathologic lesions when they were left in for ten minutes whereas they failed repeatedly to cause the slightest pathologic change when left in for periods of five and seven minutes respectively. It may be stated then that the maximum concentration of mercurochrome tolerated by the tissues of dogs when injected into the emptied vessels of an extremity was found to be 0.0033 per cent when retained for a period of seven minutes.

treated. The treated and untreated legs showed no marked differences in the rate of their improvement. In one case only did there seem to be a positive advantage in the use of the mercurochrome solution.

Although the tissues of the infected extremities were perfused with a maximum tolerated concentration of mercurochrome, it is evident from a comparison of the results obtained that such a solution has no definite antiseptic value when thus employed in the treatment of experimentally produced local infections.

#### DISCUSSION

The original method described above in detail constitutes an excellent technique for testing tissue tolerance. While we have employed it only for testing the tissues of an extremity, yet it may be used for testing the tissues of any organ possessing a single blood supply. The injection can be interrupted at any moment in order to reestablish the current of arterial blood and thus to sweep into the general blood stream all the dye or other substance except that which has become fixed in the tissues. The resulting concentration in the blood stream is so small as to make its effect negligible. Thus the time of tissue exposure is controllable. The error due to tissue dilution may be assumed to be a constant one. Although we have only tested mercurochrome, yet the method lends itself readily to the testing of any substance.

So accurate are the end-points obtained by use of this technique that we feel it fixes a true tissue tolerance point for any substance.

It is interesting to compare the concentration produced in the blood stream by injecting the average dose of mercurochrome in an average man with the maximum concentration tolerated by the dogs in our experiments. Young and Hill<sup>1</sup> advocate an intravenous injection of 5 milligrams per kilogram of body weight in fulminating human infections. This in the average adult would amount to a dose of 30 cc. of a 1 per cent solution. Regarding the total blood volume in the human as roughly one-thirteenth of the body weight, the vessels of a man of 150 pounds would contain 5230 grams of blood. If one introduces into this volume 30 cc. of a 1 per cent mercurochrome solution, there would result immediately after injection a concentration of 0.0057 per cent. Our maximum tolerated concentration of 0.003 per cent approximates this when one considers how rapidly the strength of the mercurochrome must be reduced through the single factor of excretion when the dye is injected into the general circulation, a factor which is not present in the tests above recorded.

Concerning the treatment of local infections with mercurochrome, it may be said that the lesions produced were of a local character with no evidence of septicaemia, and therefore were favorable for study by the particular method employed. The fact that the treatment was found not to be effective when applied to abscesses, cellulitis and gangrene of the extremities is conclusive proof that the highest possible concentration tolerated by the tissues was insufficient to check the progress of these experimentally produced infections.

<sup>1</sup>Young and Hill. Arch. Ped. 1927, vol. 172.

## SUMMARY

An original perfusion technique is described for determining the tolerance of the tissues of an extremity or of other organs to antiseptic solutions

This technique has many advantages chief among which are (a) elimination of all constitutional reactions (b) accurate end points, and (c) easy performance of simultaneous control experiments

Employing this technique in 20 experiments upon dogs the maximum tolerance of the tissues to mercurochrome 220 soluble was determined to be 0.0033 per cent for exposures of seven minutes. A ten minute exposure to the same concentration produced gross pathologic lesions. Concentrations of 0.0065 and 0.014 left in for five minutes produced severe functional disturbances while those of 0.350 and above were found to produce gross pathologic lesions

The antiseptic value of mercurochrome was determined by the treatment of experimentally produced local infections of the extremities with concentrations of the dye determined by the above technique to be the maximum tolerated by the tissues

Eleven out of twelve local infections in dogs treated with mercurochrome under these conditions showed no definite therapeutic advantage in its use when the progress of the treated lesion was compared with that of a similar untreated infection

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## THE PATHOLOGY OF THE THYROID GLAND\*

By JOHN W. GRAY, M.D., NEWARK, N. J.

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THYROID diseases are not common in the region where I have studied them, but I have reached certain conclusions which may be of value to other pathologists who work under similar conditions and are called upon to classify goiters and to correlate the laboratory and clinical findings

I have found elaborate pathologic classifications confusing and unnecessary. The thyroid diseases which I encountered in examining five hundred specimens were classified as follows:

Diffuse colloid goiter	51
Adenomatous goiter	264
Simple	221
Hyperfunctioning	43
Cyst of the thyroid	-
Exophthalmic goiter	165
Chronic thyroiditis	3
Carcinoma of the thyroid	15

Diffuse colloid goiter is now rarely excised and most of the 51 specimens of this type which I examined were received several years ago. These

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goiters were symmetrical and two to five times the size of normal thyroids. On sectioning I found they presented a granular or finely honeycombed appearance. Many contained multiple tiny colloid cysts and in a few there were large cysts which contained colloid or fluid substance. Microscopically the acini were irregularly distended by normal or degenerated colloid and the lining cells were cuboidal or flattened. Occasionally atrophic epithelial spurs projected into the acinar lumina, an evidence of preexisting hyperplasia. In several specimens hypertrophic areas were found throughout the gland. These areas either were remnants of the parenchymal activity which always precedes the development of colloid goiter or were a part of the hyperplastic reaction which may repeatedly occur in colloid goiter. In either case the cell hypertrophy was probably the result of iodine deficiency as shown by Marine<sup>1</sup> and was influenced by the demands of the organism for an increased amount of thyroxin. It is possible that in case the demands had been large and continuous a physiologic compensation in the form of colloid goiter would never have taken place and that the activity would have gone on until atrophy from exhaustion occurred. Colloid goiter, then, is a gland which has adjusted itself to carry on an increased amount of work without hypertrophy of the parenchyma cells or is a gland which is in a resting state following increased demands upon the organ.

More than one half the goiters that I have studied were adenomatous in type. They were of particular interest because of their neoplastic character, their slow growth from abnormal structures, the great variety of growth and degeneration which they presented and because malignancy of the thyroid could frequently be traced to adenomatous tumors.

The adenomatous goiters were usually nodular, encapsulated and multiple. However, not infrequently, the growth was single and large in size. Occasionally the gland was symmetrically enlarged with small tumors embedded in the thyroid tissue. Several specimens were diffusely adenomatous, grossly resembling colloid goiter.

There are two types of adenoma, the fetal and the adult. The former contains no colloid and probably develops from so called "fetal rests," the latter contains a large amount of colloid and develops from fetal adenomas and possibly from normal glands. The structure of the fetal thyroid consists of an undifferentiated mass of embryonic cells which later form acini. Up to puberty the interacinar parenchyma remains fairly abundant and even in adult life solid masses of cells persist. A compensatory growth of these "rests" may be initiated by hyperactivity of the gland but the growth is so slow that a nodule in the thyroid may be first noticed five or ten years later.

In adenomas studied by me, the fetal type predominated. It and the adult adenoma frequently occurred together in these goiters and both types of growth were also found in individual tumors. The typical fetal adenoma was sharply defined, grayish in color, dense in consistency and small in size. The adult or colloid adenoma was encapsulated, yellowish in color and soft in consistency.

The larger adenomas, because of inadequate and lacunar type of blood supply, showed gross evidence of hemorrhagic and degeneration. This ranged

from slight central softening with small hemorrhages to large degenerative cysts with fibrous replacement and calcareous deposits. Cysts were single and multiple, small and large and contained semisolid or fluid products of blood, colloid and tissue degeneration.

Microscopically fetal adenomas were composed of tiny closely packed acini which contained no colloid. Others which I inferred were of longer standing, showed all stages of acini development. Acini of the typical adult adenoma were lined by multiple layers of small cuboidal cells and contained a normal amount of colloid. In colloid adenomas the acini became distended and, except for adenomatous areas differentiation from diffuse colloid goiter would have been difficult. In some specimens adult acini were found in the center of the growth and fetal structures toward the periphery rather positive evidence of the development of the adult type from the centrifugal growth of fetal adenoma. Slight or moderate lymphoid infiltration was present in a few adenomas. In degenerated tumors new acini had developed in blood masses forming secondary adenomatous growth. Blood clot had undergone fibrous and hyaline transformation and in rare instances epithelial structures had grown into cysts forming a papillary cystadenoma. Blood pigment, degenerated colloid and cholesterol crystals were found in cysts and in the fibrous walls of these cysts there were primary or regenerated adenomatous glands and calcareous deposits.

Adenomatous goiters were further classified in regard to their functional activity. Of 43 diagnosed as hyperfunctioning adenomatous goiters 21 were so placed only through clinical data while 22 showed cell hypertrophy either in the tumor or in the surrounding thyroid tissue or both. The clinical group could be explained only upon the assumption that there was an increased bulk of adenomatous secreting glands in such goiters. Pathologically the hypertrophic group could not be distinguished from exophthalmic goiter but clinical data again played a part in the classification for although the basal metabolism was high in some of these cases none of them showed evidence of thyroid dysfunction.

A separate heading was made for cystic goiter because of two large thin walled cysts containing clear fluid in which the absence of gland structure made it impossible to trace their origin.

The group of 165 exophthalmic goiters presented but few difficulties in the laboratory diagnosis. They varied in size from that of the normal gland to many times that size but for the most part were only moderately enlarged and retained the normal shape. They were firm in consistency. The microscopic findings were also characteristic. There was extensive epithelial hypertrophy. The acini were irregular in size and shape and were lined by a single layer of tall columnar cells which usually formed infoldings and papillary projections into the lumen. The cell nuclei were enlarged and many abnormally placed. This epithelial hypertrophy involved not only the acini but also the interacinar parenchyma cells. Some glands contained no colloid while others contained varying amounts. The microscopic picture was changed by the administration of Lugol's solution the cells were less hypertrophic, colloid was more abundant and some specimens were so altered that wide areas

resembled colloid goiter. Although it was impossible to estimate the degree of hyperthyroidism in such glands, it was always possible to establish the diagnosis through persistent hypertrophic areas. In almost all of the exophthalmic goiters the vascular stroma was infiltrated by lymphocytes and in some of them there were lymphoid deposits containing germinal centers.

An explanation of the etiology of exophthalmic goiter is not simple and is largely hypothetical. This thyroid disease is not common in some countries where goiter is most common, which suggests that the individual reaction as well as the stimulus should be considered. Psychic shock immediately preceded the onset of exophthalmic goiter in several cases which I have observed. Plummer<sup>2</sup> advanced an attractive theory of hyperfunction plus dysfunction as the cause of Graves' disease. In support of this two-product theory Haines<sup>3</sup> reported a case in which myxedema was produced by the administration of Lugol's solution and the phenomena of exophthalmic goiter existed when it was not administered.

Three of my specimens were classified as chronic thyroiditis because inflammatory processes predominated. The reaction consisted of lymphocytic infiltration and fibrous production. In two the parenchyma was definitely atrophic, and they represented to me the atrophy of exhaustion following continuous hyperfunction of the gland. The slight evidence of inflammation found in colloid and adenomatous goiters, the marked lymphocytic infiltration so common in exophthalmic goiters, and the productive changes in "burned out" goiters, indicated a relationship of stimulus, hypertrophy, and atrophy which might be explained through infection as suggested by Cole and Womack.<sup>4</sup>

Carcinoma occurred in fifteen, or 3 per cent, of my thyroid cases. Seven were malignant adenoma, 2 papillary adenocarcinoma, one scirrhus carcinoma, and 5 undifferentiated adenocarcinoma. The malignant adenomas were encapsulated and solid or solid and cystic. In two specimens of early malignancy the gross appearance was that of fetal adenoma. The structure was essentially adenocarcinomatous although great variation occurred in the development of acini and wide undifferentiated areas resembled solid carcinoma. Of the two papillary adenocarcinomas one developed in a large cyst while the other was a nodular tumor. The latter was of more malignant grade than the former as shown by the history of rapid growth and by the microscopic examination. In the former neoplasm the papillary and alveolar structures were for the most part lined by columnar cells while in the latter the cells were polyhedral in shape, contained large hyperchromatic nuclei, and undifferentiated cell groups invaded the stroma. The scirrhus carcinoma was a very firm irregular growth which had been present for several years and was adherent to the surrounding tissues. Microscopically it resembled scirrhus carcinoma of the breast and the extensive fibrosis had apparently acted as a protective factor in the malignant advancement of the tumor. The 5 undifferentiated adenocarcinomas were types frequently termed solid carcinoma. Four of these were single firm tumors of considerable size the fifth was small and hard. They were composed of solid groups of atypical, deeply stained cells which infiltrated the capsules. Metastasis occurred in 4 of these cases.

It was impossible to determine whether these undifferentiated carcinomas originated in adenomatous or normal thyroid tissue. The grade of malignancy of my thyroid carcinomas except the sarcomatous type, paralleled the degree of differentiation of the tumor cells.

In this attempt to simplify the pathological classification of thyroid diseases I should suggest in conclusion that our understanding of thyroid pathology demands special standards dependent upon the development, structure and function of this highly specialized gland and that the wide variations in the functional activity and structure of the gland in different physiologic states may explain variations which occur in different parts of this and other countries.

#### DISCUSSION

*Dr Wm C MacCarthy*—As one of the founders of this Society I want to say something to those who have been presenting papers. Two gentlemen apologized a little bit. I used to go to a certain society where there were older members present and was almost afraid to call my soul my own. Dr Gray happened to think how many goiters I had seen and he apologized. I want to leave this little tradition with you. The Society was for the purpose of the young man who thinks. It makes no difference whether he had one case or fifty thousand, let him think about what he sees, let him draw his conclusions and come here and speak them. I never shall forget how uncomfortable I have been in certain meetings. I think that is one of the reasons I helped organize this one. We want to encourage young people. This is a young people's Society. We want that one person that is right. We will never get that if we apologize and if older people are given preference. I have seen in the paper the elaborate preparations made for flights across the Atlantic. I feel this very much myself. I still feel uncomfortable in the presence of that old Society. Don't ever let us get into that state of mind.

*Dr Philip Hillout*—We frequently get thyroids for diagnosis in the laboratory, and we are often at a loss for proper classification. We are not able to pigeonhole them so easily as they are in the textbooks. I should like to get some suggestions from Dr Gray as to how he classifies them. There is a need for a uniformity of terminology among pathologists. What constitutes chronic tonsillitis or chronic appendicitis? I do not like the term toxic thyroid. I cannot tell whether it is toxic or not. I would be very glad to hear a discussion on the part of practical pathologists as to how they solve the question.

*Dr George T. Caldwell*—In some of the exophthalmic goiters there is a diffuse infiltration of lymphoid cells. I was surprised to find that this proliferation of lymphoid tissue became the predominant feature to such an extent that the epithelial cells in many of the acini were completely replaced by lymphoid tissue. One could feel sure this was lymphoid tissue from the presence of definite lymph follicles. The cells in the central portions of these follicles are larger and more lightly stained. In some of these thyroids the epithelial structures are nearly completely replaced by these large lymph follicles. Along with the disappearance of the epithelial cells however there usually goes a very marked proliferation of fibrous tissue of the gland. There has been relatively little said about these thyroids in which the epithelial structures are replaced by lymphoid tissue. I wonder if they are common in Dr Gray's section of the country.

*Dr C I Owen*—I had the same problem that these men have been having. I worked with a thyroid surgeon. Between the two of us we developed a classification we both understood. We do not recognize Graves' disease; we call that marked hyperplasia. We recognize colloid goiter which would not be operated, the adenomas of colloid type, the fetal type, the various tumors and inflammations. As for the lymphoid proliferation I see that quite commonly being in the thyroid belt. It is found in people who are members of the situs lymphaticus group.



*Dr Robert A Keilty*—The generally accepted surgical classification of thyroid glands from the pathologist's point of view is rather unsatisfactory. The term "toxic adenoma" from the clinical viewpoint may adequately meet the needs of the surgeon but when carefully studied pathologically, these glands may show no evidence of tumor but a well marked reaction explained on inflammatory grounds. There is a great need for a revision of thyroid nomenclature with a more careful and critical study of the actual pathology present in the glands fitted to the clinical picture. This is especially true in the nodular types of thyroid which are so universally called toxic or multiple adenomas when pathologically they show none of the structure of tumor as we understand it elsewhere in the body.

In 1921 I briefly reported bacterial findings in a series of thyroid glands removed surgically and since that time I have studied the question from the inflammatory and focal infection viewpoint. The presence of foci of round cells in the interstitial structures is, I believe, an inflammatory manifestation of bacterial retention in many cases. This corresponds to the lymphocytic hyperplasia of chronic inflammations and as such many thyroids are to be considered from the standpoint of chronic thyroiditis.

The surgical removal of the thyroid gland per se is not by any means the last word in goiter therapy.

*Dr John H Gray* (closing)—I think Dr McCarty and others for their discussion of this paper. Although conclusions from a limited amount of material may be of value I should emphasize the importance of first becoming familiar with extensive authoritative studies on the subject. In answer to Dr Milkowitz' question regarding classification I agree with Dr Owen that it should be as simple as possible, that it is much easier to correlate the laboratory and clinical findings under a few headings than otherwise. I admit that exophthalmic goiter is not a scientific pathologic term, nor is it a good clinical name since it combines two symptoms of a disease both of which may be absent. However, if exophthalmic goiter and toxic adenoma are more readily understood by the surgeon than parenchymatous hypertrophic goiter and hyperfunctioning adenomatous goiter, I have no objection to using such terms. Dr Caldwell's question regarding the classification of goiter showing extensive lymphoid infiltration is easier to answer than to explain. It is commonly present in exophthalmic goiter but seldom present in adenomatous goiter. In three of my specimens the lymphoid elements predominated to such an extent that a diagnosis of chronic thyroiditis was made. Dr Keilty touched upon infection as an etiologic factor in the production of goiter. I mentioned the preliminary report of Drs Cole and Womack and consider it the most important contribution we have on the subject.

#### REFERENCES

- 1 Marne, D. Special Cytology 1 560 562, 566 568, Paul B Hoeber, New York, 1928
- 2 Mayo, C H, and Plummer, H S. The Thyroid Gland, Beaumont Foundation Lectures, St Louis, 1925, C V Mosby Company
- 3 Haines, S F. Exophthalmic Goiter and Myxedema. Report of a Case. Endocrinology 12 No 1, 55 58, 1928
- 4 Cole, W H, and Womack, N A. The Thyroid Gland in Infections. Preliminary report, J A M A 90 No 16, 1274 1276, 1928

## HENOCCH'S PURPURA DUE TO FOOD ALLERGY

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ABOUT a month after the reception of the study of this case, my attention was called to a report of three similar cases by Alexander and Everman.<sup>1</sup> The subject and its literature are so well covered by them that further comments would be superfluous beyond adding the report of a confirmatory case of this rather unusual puzzling clinical entity with the obvious suggestion of the necessity of study from an allergic point of view of chronic hemophilia and purpura.

Mrs. L. M. J., aged forty-nine, married twenty-five years, no pregnancies, gave a negative family history except that one nephew was a bleeder. No known allergy in the family.

**Past History.** Acute nephritis twenty years previously requiring medical supervision about one year. No recurrence. Two uterine curettages, seventeen and fifteen years ago for fibroids and polyp. No recurrences. The operations were done to correct prolonged and excessive menses. Menstrual history normal the past fifteen years and never affected by the present condition. Had one attack of intercostal neuralgia with herpes twelve years ago. Severe urticaria persisted through one summer thirty years ago and again twenty-two years ago. There have been a few attacks since of no great severity. The patient has never noticed any connection between the urticarial and the purpuric eruptions. No other illnesses have occurred except occasional acute infections of the upper respiratory tract. No hay fever or asthma. She has always been susceptible to bruising from slight traumatism. Has always had frequent nosebleeds of considerable difficulty to control. Gums have always bled easily. Two teeth extractions, however, were done within the last four years with little or no difficulty from this source.

**Present History.** The first eruption of black and blue spots occurred on the upper arms and thighs eight years ago, lasting two weeks with exacerbations and retrogressions. Since the initial attack a few new black and blue spots have occurred every three or four days on arms, thighs and lower legs, only once on the face and rarely on the abdomen or chest. The largest number of comparatively large areas, varying in size from that of a silver dollar to the palm of the hand, remembered at any one time was about twenty. Individual purplish areas have usually lasted a week or ten days, then fading to yellow, disappearing completely in about a month's time. She has never had any joint symptoms, but has usually been able to foretell an eruption by noting a few hours or days of muscular soreness where the eruption would subsequently appear.

Since the initial eruption nosebleeding has been very frequent, occurring at times daily and occasionally two or three times daily. Three years ago there were daily nasal hemorrhages over a two-months' period. On one occasion she bled for six hours. For the last three years, she has had no bleeds of considerable severity at least twice a week. There has been one nasal hemorrhage six years ago.

Digestive disturbances have occurred for twenty or twenty-five years, taking the form of intense abdominal pain and vomiting. No diarrhea but considerable belching. There was never any jaundice. The digestive disturbances would usually be accompanied by either no bleeds or the black and blue skin eruptions or by a few urticarial lesions.

The initial etiologic diagnosis of the nasal hemorrhages as purpuric was made by Dr. W. A. Hitchcock, a nasal specialist of Boston, some four years previous. Several thorough gastrointestinal examinations have been negative.

This case was first seen by me October 10, 1927, the morning after a severe nasal hemorrhage, accompanied by a few scattered urticarial wheals and a few small petechial areas on the arms and legs. General physical examination was negative. Nasal examination by Dr C J Bochs was negative. Special examination by Dr W E Nesbit, including blood, was negative.

On account of the urticarial history and the fact, that at that time it was noticed that a number of my seasonal hay fever patients were showing bruised areas from trifling traumas, an observation since not infrequently confirmed in my experience, the possibility of an allergic basis was at once considered. Skin tests by the cutaneous method over a number of days were then done to approximately two hundred antigens. During the course of testing, intradermal tests were done to the ragweeds and grasses, using 1:50 extracts, securing definite positive reactions to the grasses. These special pollen tests were followed almost at once by generalized itching over the entire body lasting for twenty-four hours, but not producing any other symptoms or any skin eruption. Skin tests were slightly positive to onion, shellfish and the split proteins of wheat.

**Subsequent History.** Since October, 1927, she has been under the following antiallergic precautions. First, restriction of wheat and cereals, except for toasted bread, second, avoidance of fish and onion. Since this type of diet was suggested, the patient has noticed that either skin eruption or nosebleed or digestive disturbances, or all three, can be brought on by eating flour thickened gravies, sauces and soups, and she herself now considers this factor, and onion her principal trouble.

At this writing, March, 1929, this case has been under observation one and a half years. There have been in this period six small nasal hemorrhages of only a few moments' duration, about six light attacks of urticaria of a few hours' duration, and almost none of the petechial eruption. These relapses have invariably followed unavoidable breakage of the ordered dietary restrictions. Previous to this study, this case had always been a heavy bread eater and a good liver generally.

About the end of April, 1928, she had three or four severe digestive disturbances lasting several days, accompanied by considerable loss of weight, a few hives, two small epistaxes and a considerable amount of purpura, one attack ascribable to the usual thickened flour gravy, the other three attacks being due to Boston brown bread. Abdominal pain was intense in each instance, simulating typical acute cholecystitis. Gastrointestinal examinations at the time at the Winchester, Massachusetts, Hospital by Drs R G Vance and M J Quinn were negative.

This patient was seen again February 22, 1929, reporting continued adherence to dietary restrictions without medication of any kind, and absolute freedom from previous symptoms during the past nine months, the longest period of well being in twenty-five years.

She was retested at that time to all common foods, showing slight reactions to banana, strawberry, lamb, crab, halibut, mackerel, codfish, pear, sweet potato, and a strongly positive reaction to peanut, which she knows annoys her. Definite purpuric areas from 3 cm to 5 cm in diameter were noted from six to eight hours later, surrounding the skin tests to plum, barley, halibut, mackerel, lima and string bean, probably traumatic and not specific. At no time did her actual etiologic factors show any but the slightest possible positive reactions.

#### REFERENCE

1. Alexander, H L, and Evermann, Charles H. Food Allergy in Henoch's Purpura, Arch Derm and Syph 16:322, September, 1927.

## ASTHMA DUE TO HOUSEHOLD ARTICLES\*

### REPORT OF NINETEEN CASES DUE TO DUSTS FROM MATTRESSES

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ALTHOUGH it has long been recognized that the majority of asthmatics have symptoms due to hypersensitiveness to some substance, the ingenuity of the physician is frequently taxed to the utmost to determine the origin and nature of the exciting agent. Innumerable substances have been implicated from time to time. Many of these arise from animal emanations and from the products used in the manufacture of various household articles. Kern<sup>1</sup> first called attention to house dust as a cause of asthma and Cooke<sup>2</sup> demonstrated sensitivity to dust in over 40 per cent of patients whom he tested. Rowe<sup>3</sup> found 42 per cent of one hundred and sixty-two patients sensitive to house dust extracts and felt that this substance, house dust, was frequently of importance as a cause for symptoms.

For the past three years it has been our custom to test all patients with extracts made from dusts collected from various articles in their environment. The dusts are collected as follows: The cloth bag is removed from a vacuum cleaner, the cleaner is operated for a moment to free the working mechanism of any dust which may be present and a sixteen pound paper sack is tied on in place of the cloth bag. A sample of dust is then taken from the mattress of the patient's bed at least a teaspoonful is required. The bag is removed, labeled, a new bag is tied on and another sample of dust is obtained from the pillows. Other samples of dust are collected from each mattress and set of pillows from each rug, from the overstuffed furniture, and from any other articles in the home which may harbor dust. Each sample is placed in a wide mouthed bottle and covered with Bernton's fluid at room temperature for forty-eight hours. The extracts are filtered and are used by the scratch method.

Among two hundred patients who have been tested to dusts collected and prepared in this manner nineteen have been found who gave large reactions to extracts of the dust collected from their own mattresses but who did not react to cotton or kapok of which the mattresses were made. In each instance complete relief was produced almost like magic by discarding the offending mattress and the substitution of a new one. However in three cases attacks recurred after free intervals of from four to six months whereupon positive skin tests were again obtained to extracts of the new mattresses. It was found however that recurrences could be prevented if the mattresses were covered with some impervious material either rubber sheeting or Dupont's satin fabricoid.

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Studies are being made to determine the nature of the sensitizing material in these cases. It is probable that mattresses become infected with molds to which patients become sensitized, as has been pointed out by W. Storm van Leeuwen.<sup>4</sup> This is probably what has occurred in these cases, since mattresses, the dust extracts of which give no reactions and which produce no symptoms in patients when new, do cause trouble after several months' use. The following cases illustrate these points:

N. B., a married woman of thirty-four years, has had asthma of a perennial type for the past fifteen years. During this time the longest free period was a six months' period which was spent away from home. There were no clues in the history pointing to the etiologic factors.

Examination revealed a well-developed woman of thirty-five years with moderate chronic bronchitis and slight emphysema. Blood and urine studies revealed no abnormalities. Routine skin tests, using food, epidermal protein, pollens, and vaccines, two hundred tests in all, were negative. A reaction was obtained to an extract of dust from the mattress on which she slept. No reaction was obtained to cottonseed, or to kapok.

Treatment consisted in discarding the offending mattress and completely renovating the bedroom. This was done on January 29, 1927. The attacks ceased within a week and have not recurred to date, seventeen months later.

J. E., a boy of thirteen years, has had attacks of asthma occurring about once a month for seven years. There had been eczema during infancy, and frequent attacks of bronchitis with wheezing, beginning in early childhood and continuing until the onset of the asthmatic attacks. The longest period of freedom from attacks was two months, but there was some rhinitis almost daily.

Physical examination revealed no intrinsic causes for the attacks. Routine skin tests using 200 substances revealed positive reactions to several fruits, to beef, and to spinach. Removal of these foods from the diet had no effect on the symptoms. Tests with autogenous dust extracts revealed a strongly positive reaction to the dusts from the patient's mattress and from aavenport in the living room.

The substitution of a new cotton mattress for the old one, after a complete renovation of the bedroom, produced relief from the asthma for a period of six months. During this time there was no rhinitis.

After the six months' interval, sniffing and other symptoms of allergic rhinitis recurred, and were followed, within two weeks, by a mild asthmatic seizure. A rechecking of the environment revealed a hypersensitiveness to dust collected from the new mattress.

This mattress was covered with rubber sheeting to preclude the escape of dust. No further attacks have occurred during a period of observation of one year.

#### REFERENCES

1. Kern, A. Dust Sensitization in Bronchial Asthma, *Med. Clin. N. A.* 5: 751, November, 1921.
2. Cooke, R. A. New Etiologic Factors in Bronchial Asthma, *J. Immunol.* 7: 147, 162, March, 1922.
3. Rowe, A. H. House Dust in the Etiology of Hay Fever and Asthma, *Arch. Int. Med.* 39: 498-507, April, 1927.
4. Van Leeuwen, W. Storm. The Pathogenesis and Therapy of Bronchial Asthma, *München med. Wchnschr.* 73: 559-603, April 9, 1926.

# AN UNTOWARD EFFECT OF BARIUM CHLORIDE IN PRODUCING SHORT RUNS OF ABERRANT VENTRICULAR BEATS\*

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FOLLOWING its introduction into the therapeutics of A V heart block by Cohn and Levine,<sup>1</sup> barium chloride has come to be used fairly extensively to increase the idioventricular rhythm of this disturbance and so to prevent the occurrence of synopal and convulsive seizures.

Its clinical use was suggested by the results of animal experiments by Rotberger and Winterberg in a paper in which they showed that the drug increased the irritability of the ventricles as shown by the occurrence of isolated ectopic ventricular beats and even short runs of paroxysmal tachycardia.

Among those who have reported upon the use of the drug in clinical heart block, in addition to Cohn and Levine<sup>1</sup> are Herrmann and Ashman,<sup>2</sup> Heard, Marshall and Adams<sup>3</sup> and Strauss and Meyer.

On the whole these reports are favorable toward beneficial effects of the drug. Its use, moreover, has been thought to be entirely safe within the limits that the drug is usually prescribed. In one of their cases Herrmann and Ashman<sup>2</sup> used five times the usual therapeutic dose with no other untoward results than nausea and vomiting.

In a recent experience with barium chloride we encountered an untoward effect to which we feel attention should be called the more so because the same disturbance occurred in the case of Strauss and Meyer although they did not stress its potential danger. The disturbance to which we refer was the occurrence of runs of irregular ventricular tachycardia a mechanism not far removed from ventricular fibrillation.

A resume of the essential history and course of the patient follows.

A white woman fifty-three years old had been under our observation either as a private or hospital patient continuously for six years. During this time she had had five attacks of complete heart block none of which lasted for more than two months. During the periods of slow rhythm she was breathless on the slightest exertion, very easily fatigued and showed a slight but generalized edema. Between these attacks she went for periods longer than a year with normal sinus rhythm and without disturbance other than slight fatigue and breathlessness on exertion. Examination showed only moderate evidence of circulatory disease except for the periods of heart block. The peripheral arteries were only moderately sclerosed though the blood pressure was constantly elevated the systolic varying between 168 and 141, and the diastolic between 100 and 80. The heart itself was no more than slightly enlarged the left base being 12 cm. and the right 3 cm. and the poststernal dullness 6.5 cm. Auscultation revealed only a systolic apical murmur. At the apices of both lungs there was an old inactive tuberculous process.

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The first three attacks of heart block disappeared spontaneously after having lasted various lengths of time up to two months. In the fourth attack (January, 1927) she was given barium chloride for the first time and left the hospital after ten days with a normal sinus rhythm on a maintenance dose of 20 mg twice daily. On this she continued to have a normal sinus rhythm with the exception of some brief periods of 2:1 block, lasting for only a few minutes or, at most, hours until eight months after her last discharge from the hospital when she developed complete A-V heart block which continued until her death in December, 1927.

During this last period of heart block, while still under the care of one of us in her home, the dose of barium chloride was increased to 20 mg four times a day. After three days this resulted in the appearance of a very disturbed ventricular rhythm, which, without an electrocardiogram, was interpreted as being due to short paroxysms of ventricular tachycardia and led to the discontinuance of the drug. On December 5, 1927, after hospitalization had been instituted, the drug was again begun in a dosage of 20 mg four times a day and continued for four days. It was then discontinued because of the return of frequent short periods of a very disturbed and irregular ventricular tachycardia (Fig 2). The disturbance was present in about the same degree on the day after the drug was discontinued. On the second day occasional isolated extrasystoles were still noted, though no runs were present. Almost exactly seventy-two hours after the barium was discontinued, the patient died very suddenly. Two hours before death the pulse rate was 36 and its rhythm regular.

#### ELECTROCARDIOGRAMS

Fig 1 shows the three customary leads of an electrocardiogram taken the day before barium chloride was begun. This tracing shows complete A-V block with a usually regular idioventricular rhythm of approximately 28 beats per minute.

Depressions of the ventricular rate occurred quite frequently, bringing the rate down to 16 beats per minute for a few cycles. These alarming depressions of rate suggested the likelihood of the occurrence of periods of ventricular standstill with accompanying Stokes-Adams seizures. These never occurred (unless her sudden death was the result of such an attack), but the fear of them was one factor that led us to use barium chloride.

After barium chloride had been exhibited for twenty-four hours, the alarmingly long pauses between ventricular beats became more frequent, and even the regular and more dominant ventricular rate became slower, being 28 before and 25 after twenty-four hours of barium therapy. The auricular rate became quickened from 78 to 93 after barium. We do not regard the change in either the auricular or ventricular rate as being a barium effect but rather as a coincidence.

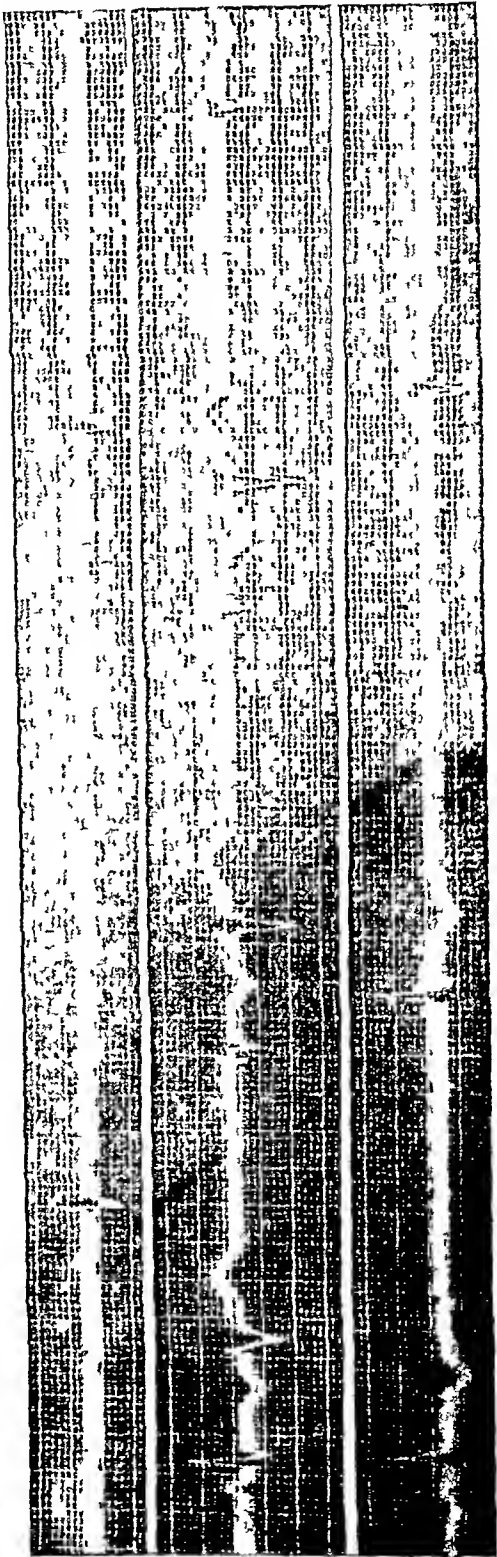
The first three strips of Fig 2 are the customary three leads taken after forty-eight hours of barium. They show the auricular rate quickened to 126 and the dominant ventricular rate increased to 30, which is slightly higher than the prebarium rate, though there are still periods in which the latter becomes quite slow. It also shows single ventricular extrasystoles coupled with preceding supraventricular impulses.

Fig 2, lower three strips, shows the electrocardiogram taken after 220 mg of barium chloride had been given in ninety-six hours. It shows the idioventricular rhythm being frequently interrupted by runs of as many as seven ventricular extrasystoles occurring at a rate of approximately 210 per minute. These beats, while apparently originating at the same focus, are quite irregu-



Fig 1—The first three strips are the customary three leads taken before barium chloride was begun. There is complete A V heart block. Time intervals one-fifth and one twenty-fifth seconds.





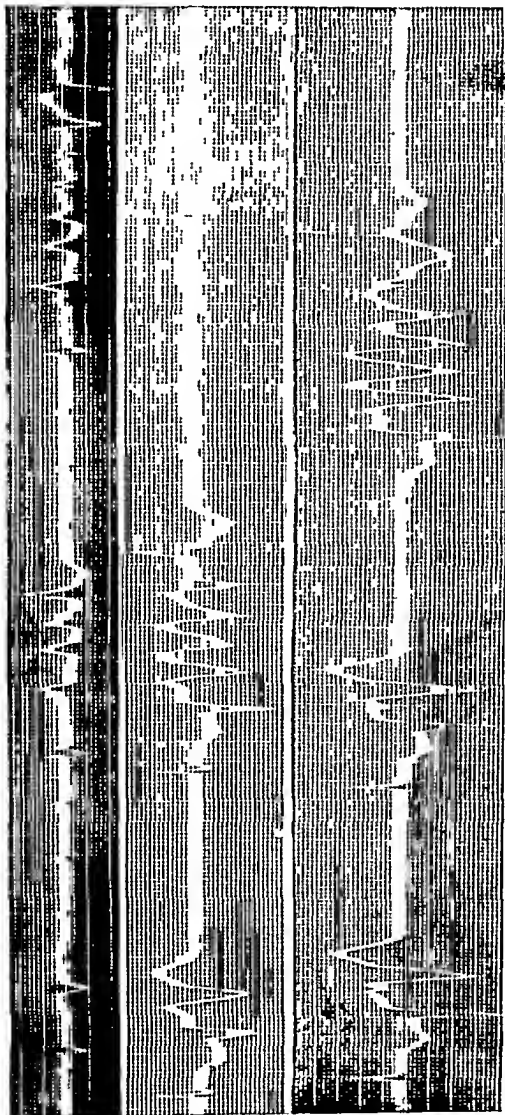


Fig. 1.—Top three strips are the tracings taken forty eight hour after the beginning of barium. The auricular rate is quickened to 1% per minute. The ventricular rate is slightly increased over the prebarium level reaching 30 per minute. Coupled ventricular extra systoles are also shown. Time intervals one fifth and one-twenty fifth seconds. Lower three strips represent a tracing taken after 0.05 g of barium chloride had been administered in thirty six hours. The normal flow-ventricular rhythm is interrupted by frequent short runs of aberrant ventricular beats occurring at a rate of approximately 90 per minute. The effective ventricular rate as a result is lowered. Time intervals one-fifth and one twenty fifth seconds.

lar in their occurrence and somewhat so in their shape. If one counts the ectopic beats, the average ventricular rate is considerably more rapid than before barium was begun. The number of effective ventricular contractions, however, was only 25 during this disturbance, whereas it was 35 before, and individual pauses between successive ventricular beats are quite as long as before barium was begun. This disturbance, near as it is to ventricular fibrillation, we regarded as an untoward effect of barium chloride and caused us to discontinue the drug.

A tracing, not presented, taken on the day after the drug was discontinued showed the same disturbance though the runs were not so long nor of such frequent occurrence. On the second day after the barium was stopped, no tracings were obtained but clinically isolated single ventricular extrasystoles were noted. The sudden death of the patient early in the morning of the third day after discontinuance of barium, prevented our determining how long the extrasystolic disturbance would continue after the barium had been stopped.

#### DISCUSSION

Lewis<sup>1</sup> has stated that one is justified in denoting runs of as many as six rapid ectopic ventricular beats as ventricular paroxysmal tachycardia. In our case runs of as many as seven ectopic beats were noted. The disturbance, therefore, can be called ventricular paroxysmal tachycardia or, at least, potential tachycardia. We do not believe that the sudden death of the patient was the result of this disturbance as the latter was definitely waning. We feel, however, that the occurrence of this disturbance on a relatively small dosage of barium chloride is worth a warning against giving the drug to the point of causing this potentially serious disturbance.

The disturbance in ventricular rhythm resulted from a dose of only 20 mg three or four times a day for four days—a total dosage of only 220 mg over a period of four days. This dosage is small, as the drug has been given by some of those who have reported upon it. Heilmann and Ashman used a dosage as high as 50 mg every four hours for six doses per day for one week, without its having caused any serious disturbance in ventricular rhythm. The results that we have noted as well as those of Strouss and Meyer, make us feel that barium chloride is not a drug to be given in even moderate amounts without careful observation.

In spite of the increased irritability of the ventricle that resulted from barium, there still continue to be long pauses between successive ventricular beats, and the actual effective ventricular rate was slower. In other words, in our case barium induced a potentially serious disturbance in rhythm without removing the threat of ventricular standstill, or improving the circulation.

Elimination of barium is apparently rather slow. In our case isolated extrasystoles continued to be noted clinically forty-eight hours after the drug was discontinued.

#### CONCLUSIONS

A case is reported which during six years of observation had four attacks of complete A-V heart-block lasting for varying lengths of time up to two months. During the third attack barium chloride may have been a factor in

restoring the sinus rhythm and preventing the onset of complete block again for eight months.

During the fourth attack of complete block which lasted four months and ended in death, barium chloride failed to increase the effective ventricular rate in doses of 20 mg four times a day for four days.

This dosage of the drug brought on a marked extrasystolic disturbance with frequent short runs of rapid ventricular tachycardia. The same disturbance in the case of Strauss and Myer is referred to.

The disturbance is reported because we regard it as potentially serious and an untoward result of barium chloride and because the drug previously has been regarded as harmless in much larger doses than were required to bring about the disturbance in this and the other reported case.

#### REFERENCES

- 1 Cohn A E, and Levine S A Arch Int Med 36 1 1925
- 2 Rothberger C J, and Winterberg H Arch f d ges Physiol 142 461 1911
- 3 Herrmann G R and Ashman R Am Heart J 1 269 1926
- 4 Heard, J D, Marshall W R and Adams F S Am Heart J 2 562 1927
- 5 Strauss S, and Myer J Am Heart J 3 328 1928
- 6 Lewis, Sir T The Mechanism and Graphic Registration of the Heart Beat, Shaw and Sons London 1925

## REGARDING THE EFFECT OF EPINEPHRIN UPON THE T WAVE OF THE ELECTROCARDIOGRAM

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THE action of epinephrin upon the form of the T wave of the axial electrocardiogram in the dog is constant when the vagi nerves are sectioned. The first effect is a negative influence upon the T wave which occupies the early part of the reaction usually before the greatest increase in the rate has occurred. It passes rapidly into the second stage of the effect a positive influence upon the T wave which persists during the time the heart rate is increased (Fig 1). An after effect of the action of epinephrin is often present reappearance of a negative influence upon the T wave which then slowly returns to its original form. The primary negative influence upon the T wave persists when the drug is injected directly into any of the cardiac chambers. If, however the drug is injected in a manner which directs its distribution to the peripheral circulation before it arrives at the heart i.e. into the aorta the first or negative effect upon the T wave is not present, and the T wave changes begin with the positive influence<sup>1</sup> (Fig 2).

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The experiments were upon 5 dogs under chloroform narcosis artificial respiration vagu nerve section and opened thorax. The epinephrin was uniformly introduced into the blood stream in doses of 0.1 cc per 10 kilograms and diluted to 1 cc with saline solution. Electrocardiograms using the axial lead (RA—LL) were taken at frequent intervals during the reaction. The total trials of the drug numbered twenty.

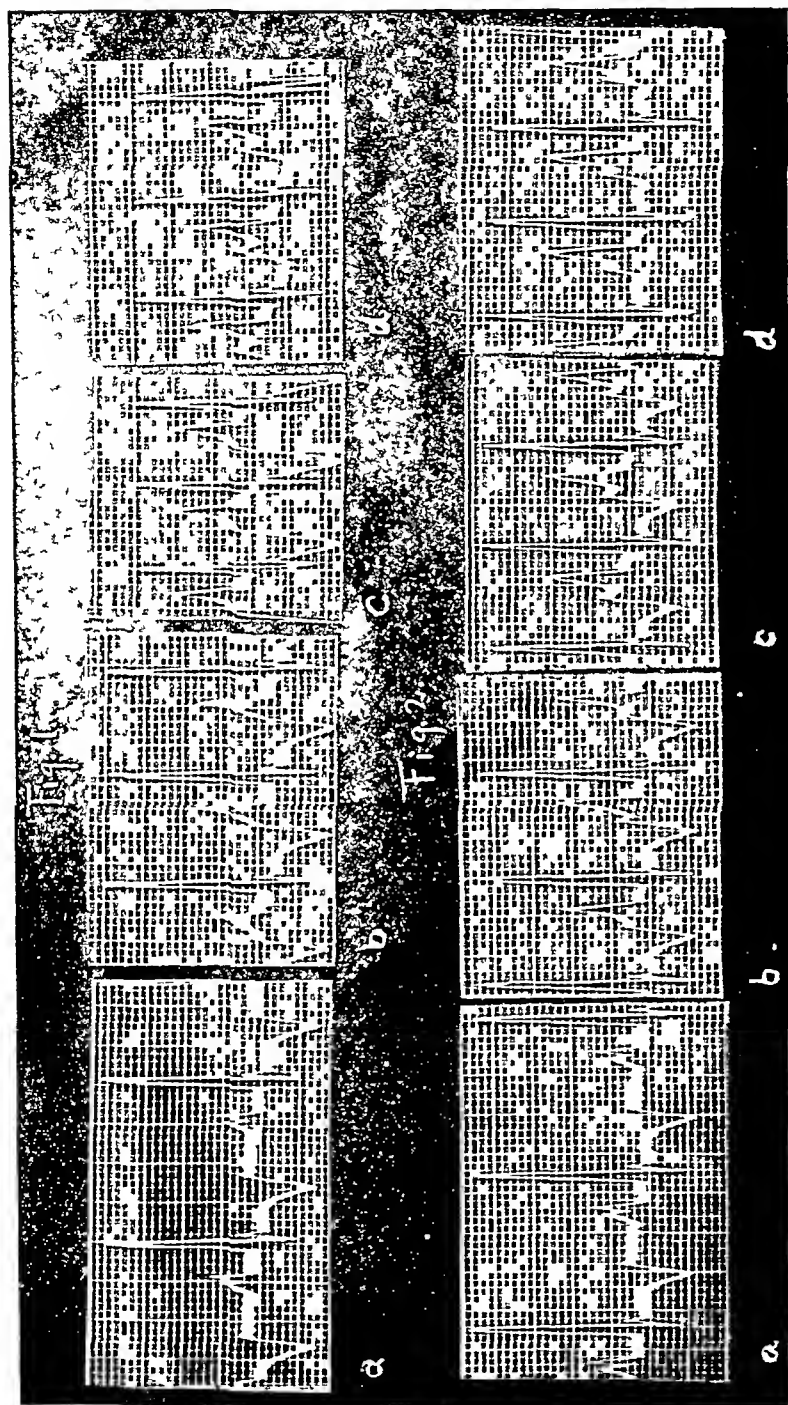


Fig. 1—Axial (RA—LL) electrocardiogram. Time in fifteenth seconds. *a* The normal curve. *b, c, d, e* Successive electrocardiograms of the tachycardia following the administration of epinephrine into the supracardiac vein.

Fig. 2—The same animal. *a* The normal curve. *b, c, d* Electrocardiograms of the tachycardia following the administration of the epinephrine by way of the arch of the aorta.

Since the effect of the adrenalin is the same whether the pulmonary circuit is first exposed to the drug or this is avoided by injecting it into one of the left cardiac chambers, the initial negativity of the T wave cannot be due to the action of the drug upon the endocardial lining of the right ventricle or its Pulm. artery tissue nor to any action it may have upon the pulmonary capillaries or lung tissues (i.e. to a changed pressure in the pulmonary circuit). Since this preliminary influence upon the T wave does not occur when the injection is made into the aorta, it is in all probability related to the earlier entry of the adrenalin into the coronary circulation, and therefore the negative influence of the epinephrin upon the T wave of the axial electrocardiogram is a direct cardiac effect of the drug, whereas the positive influence is an effect which is indirectly produced by its peripheral action. This does not however, suggest that epinephrin has a direct action upon the heart distinct from that which occurs through the accelerator nerve endings. The absence of the initial effect upon the T wave is simply due to the sudden increase of the blood pressure following the intense constriction at the periphery which tends to elevate the T wave and mask the reverse effect.

The *urea concentration* test was carried out as already described. The tests were made in the morning and the patients were allowed no food and little fluid after the preceding evening meal.

*Blood determinations* were made in the routine manner. Nonprotein nitrogen was considered normal up to 35 mg, urea nitrogen up to 15 mg, creatinine, 2.5 mg, and uric acid, 4.5 mg per 100 c.c. of blood.

Results of the examination of these 56 patients are given in detail in Table I and summarized in Table II.

#### ANALYSIS OF RESULTS

*Urinalysis*—Albumin or casts in the urine indicated the possible presence of renal disease in 60 per cent of the cases.

*Urea Concentration and Phenolsulphonephthalein Tests*—Concentration of urea was below normal in 60 per cent of the cases, while phenolsulphonephthalein was 40 per cent or less in 62.5 per cent of the cases and 45 per cent in 12.5 per cent. Of 17 cases of early or subacute nephritis (Table I, Groups 2 (b), and 4) the urea concentration test indicated the presence of disease in 16, while phenolsulphonephthalein excretion was abnormal in only 10. In other words, phenolsulphonephthalein excretion was normal in 7 patients who had other evidence of renal disease.

On the other hand, of 20 patients with predominating symptoms and signs of heart disease (Table I, Groups 2 (c), 2 (e), and 3) phenolsulphonephthalein excretion was 40 per cent or less in 15 and 45 per cent in 3 more, while urea concentration was below normal in only 9 individuals. Eleven patients with pronounced cardiac symptoms and much edema, but with little or no renal impairment, as indicated by normal urea concentration and negative urine and blood findings, showed a phenolsulphonephthalein excretion of 45 per cent or less. It appears that in many cases of cardiac disease with edema phenolsulphonephthalein excretion is a poor test of renal function, as in such cases the absorption of the dye by the peripheral circulation is greatly impaired. This is in harmony with the experience of Christian and O'Hare, who state that in cardiac passive congestion phenolsulphonephthalein excretion may be moderately low without any real kidney disease.

*Nonprotein Nitrogen and Urea Nitrogen*—In only 23 per cent of the patients was there even slight abnormality of nonprotein nitrogen, while urea nitrogen was above normal in 43 per cent. This is consistent with the statement of other observers that an increase of the chemical constituents of the blood is a late sign of renal disease.

*Creatinine and Uric Acid*—Creatinine was above normal in only 1 patient, while uric acid was above 4.5 mg. in 16 cases, 3 of these showed no other evidence of renal disease, while 4 had definite nephritis with normal nonprotein nitrogen and urea nitrogen.

Returning to the question of the value of the urea concentration test as a quantitative indicator of renal function, it may be said that calculations devised to allow for the relation of amount of urine voided (Chart I) and concentration of urea prior to the test (Chart II) to maximum urea concentration have

TABLE I  
RENAL FUNCTION TESTS IN PATIENTS

NO	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG	PHTHALIN PER CENT 130 MIN	UREA CONCENTRATION OF URINE										REMARKS
						BEFORE TEST		AFTER TAKING 15 GM UREA								
						AMT URINE CC	PER CENT	1 HR AFTER		2 HR AFTER		AMT URINE CC	PER CENT	AMT URINE CC	PER CENT	
								UREA	PER CENT	UREA	PER CENT					
1 Patients Showing no Evidence of Renal Disease																
1	21	124/84	0	12	50			98	224	60	307	Syphilis tested	Renal function			
2	20	120/80	0	13	30			66	118	55	232	Tonsillitis	Nocturia			
3	13		0	13	80			86	197	65	280					
4	20	119/80	0	15	43	42	220	44	31	46	360	Recovered from acute nephritis				
5	12	98/46	0	14	43	70	660	86	188	27	339	Constipation Syphilis Headache				
6	20	120/92	0	14	40	60	106	83	232	93	297	Tonsillitis				
2 Hypertension (a) With no Laboratory Evidence of Nephritis and no Signs of Heart Disease																
7	61	120/160	0	15	60	56	251	69	302	66	332	Nocturia ++				
8	49	226/120	0	14	85	23	119	78	344	28	300	G.I. Ringing in L. ear				
9	37	186/84	0	13	80	10	195	66	304	74	345	Nocturia ++ Slight palp & dysp				
10	42	173/115	0	15	50			48	344	40	375	Focal infection Nocturia				
11	47	160/100	0	17	47	19	138	75	231	59	315	Headache Nocturia +				

Where other constituents of the blood are abnormal they are recorded under Remarks



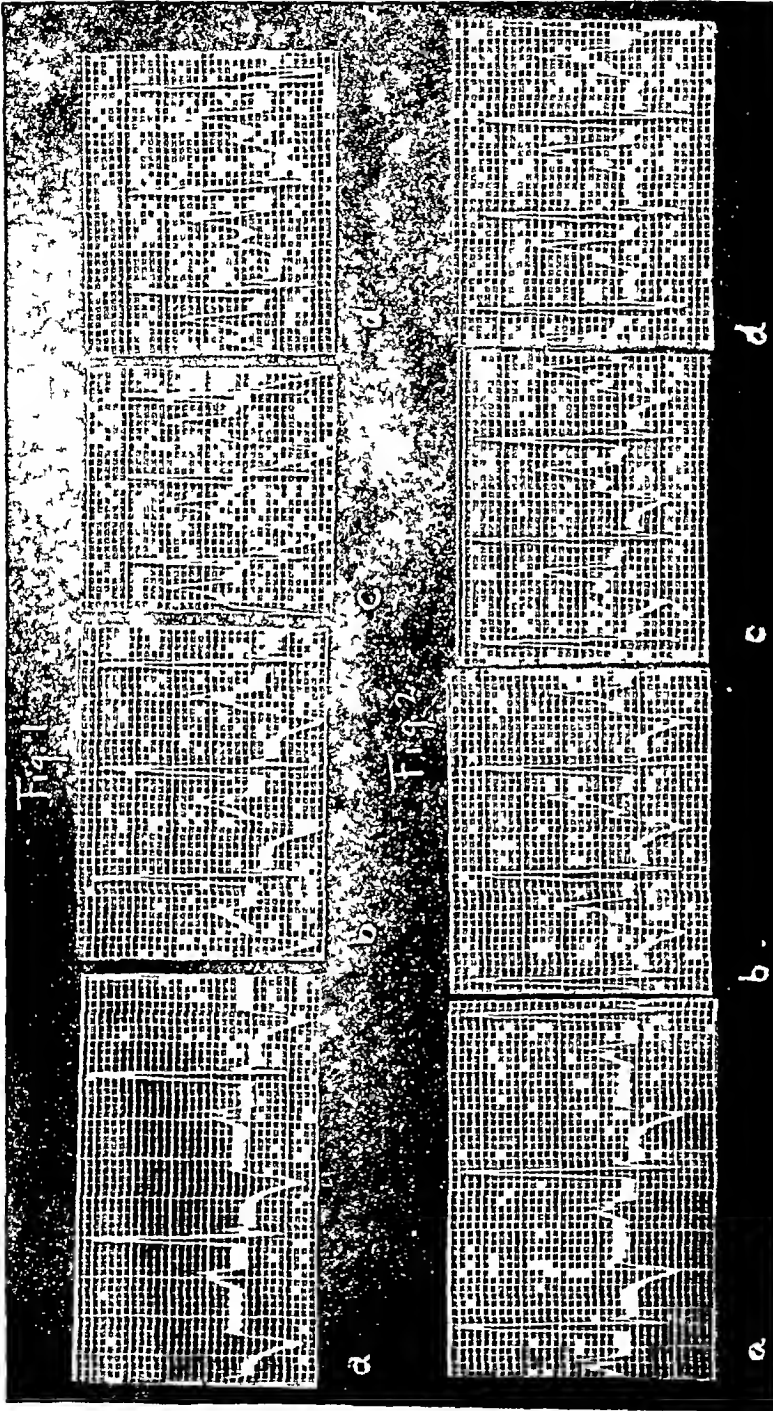


Fig 1—Axial (RA—LL) electrocardiogram. Time in fifteenth seconds. *a* The normal curve *b c d, e* Successive electrocardiograms of the tachycardia following the administration of epinephrin into the superior vena cava.  
Fig 2—The same animal. *a* The normal curve *b c d* Electrocardiograms of the tachycardia following the administration of the epinephrin by way of the trachea of the total

Maclea records excellent results with the urea concentration test in a series of more than 10,000 cases. Rabinowitch likewise reports good results. Lowen,<sup>6</sup> in a comparative study of various renal function tests, concluded that the urea test was more delicate than the phenolsulphonephthalein test and found it corresponding better with the day night urine volume ratio and specific gravity, which Christian and O'Hare<sup>7</sup> consider the most delicate test of all, than did the phenolsulphonephthalein test or blood urea nitrogen. In spite of these favorable reports the test has not been generally adopted in America. For this reason it has been thought worth while to determine the value of this test as compared particularly, with the widely used phenolsulphonephthalein test. Some observations on the relation of these two tests have been reported briefly elsewhere.<sup>8</sup> The results of renal function tests in 49 healthy students and 56 hospital patients are here considered.

#### RENAL FUNCTION IN HEALTHY INDIVIDUALS

**Methods**—All the students examined were in good health, and urinalysis was negative in each case. Tests were made in the afternoon one or more hours after a light lunch.

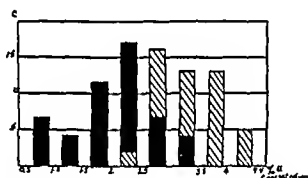


Fig. 1. The effect of the amount of urea on the urea concentration 1 hour and 2 hours after ingestion.

■ Urea Concentration 1 hour after ingestion of urea  
▨ Urea Concentration 2 hours after ingestion of urea

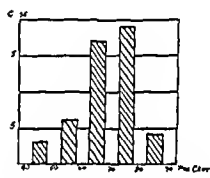


Fig. 2. Phenolthalein excretion 1 hour and 2 hours after ingestion of urea.

**Urea Concentration Test**—Blinds were somewhat restricted for two hours prior to the giving of urea as well as during the test. In order to determine the concentration of urea in urine under normal conditions the urea concentration of specimens voided just before the administration of urea was determined. At one hour and two hours following the ingestion of 15 gm of urea dissolved in 100 cc of water, urine was collected, its quantity measured, and the urea concentration determined by MacLea's technique. Urine specimens of more than 120 cc were discarded.

**Phenolsulphonephthalein Test**—A phenolsulphonephthalein test was carried out on each student within a few days of the time of performing the urea concentration test. The dye was injected intravenously in all cases and the colors matched without the aid of a colorimeter.

Forty nine students were examined. Maximum urea concentration varied from 24 per cent to 44 per cent, the average being 32.2 per cent. In only 2 instances was the urea concentration slightly below (2.46 per cent and 2.4 per

# LABORATORY METHODS

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## A STUDY OF THE NATURE OF THE UREA CONCENTRATION TEST AND ITS VALUE AS A TEST OF RENAL FUNCTION\*

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**I**N 1920 MacLean and de Wesselow<sup>1</sup> introduced a simple test of renal function based on the response of the kidneys to the ingestion of a definite quantity of urea. The concentration of urea in two urine specimens, collected at hourly intervals after the taking of urea, is determined by a chemical method requiring only two minutes in its performance, and of such a simple nature that the method is as readily available to the country practitioner as for the internist with elaborate laboratory facilities at his disposal.

Besides its simplicity many theoretical advantages may be adduced in favor of such a test as an indicator of renal function. Urea is a normal end-product of protein metabolism, and its excretion by the kidney is one of the important functions of that organ. Following administration, urea undergoes no chemical change and normally its rate of excretion is rapid and is governed by its concentration in the blood.<sup>2</sup> Conceivably the rate of absorption of urea from the alimentary tract may be delayed. However, Harrison<sup>3</sup> found normal results in 7 cases of gastrointestinal disease. Moreover, in cases of impaired circulation, as from heart failure, absorption from the alimentary tract is undoubtedly better than from the periphery. A great deal of work must be done by the kidney in eliminating urea and, as Rabinowitch<sup>4</sup> points out, the greater the degree of concentration, the greater the work per gram. By the ingestion of urea, then, the actual capacity of the kidney to do additional work is measured.

For the estimation of urea, MacLean describes a simple apparatus made with a graduated burette, a bottle, a bell-shaped vessel, and some rubber tubing. Sodium hypobromite is used for the liberation of nitrogen from the urine, and a table showing the percentage of urea equivalent to cubic centimeters of nitrogen evolved, is given. This apparatus I have found to be more accurate and easier to manipulate than the more commonly used Doremus ureometer.

The hypobromite method of estimating urea, although not perfectly accurate, is nevertheless exact enough for ordinary clinical work. Harrison,<sup>3</sup> investigating the effect of proteinuria on the urea concentration reading, found that as much as 2 per cent of albumin made no appreciable difference. Ordinary degrees of hematuria, also, had no influence on the test. Harrison gives a simple method of removing protein from the urine which can be used when large quantities of albumin are present.

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Chart I is an attempt to determine the value of the urea concentration test as a quantitative indicator of renal function. The results of all the phenol sulphonephthalein determinations have been arranged in descending order, and, in this order, the phenolsulphonephthalein percentage and corresponding maximum urea concentration in each case are plotted. It is apparent that the two curves do not coincide as they should if they were both quantitative indicators of the excretory power of the kidneys.

In the same chart the amount of urine in which the concentration of urea was measured is plotted in relation to the corresponding urea concentration. It appears from this chart that quantity of urine has a definite relationship to concentration of urea, concentration being high when the amount of urine is low and vice versa. MacLean's instructions that urine samples of 120 cc or more should be discarded recognize this influence of volume on concentration of urea. Judging by the variation apparent when even quantities of less than 120 cc are considered one is led to doubt the value of the urea concentration test as a *quantitative* indicator of renal function. It must be remembered, however, that such a conclusion is based on the hypothesis that phenolsulphonephthalein excretion indicates quantitatively the excretory power of the kidneys.

Another factor which influences the maximum urea concentration of urine following the ingestion of urea is the amount of urea normally being excreted by the individual. We have already seen that this amount varies a good deal from individual to individual. Some idea of the relationship of these two figures may be gained by determining the urea concentration in urine just prior to the ingestion of urea. In Chart II the normal urea concentration of the urine of 38 students is plotted in relation to the urea concentration two hours following the ingestion of 15 gm of urea. The two curves appear to coincide, although somewhat irregularly.

To sum up, then, both the urea concentration and phenolsulphonephthalein tests were fairly consistent in indicating a healthy state of the kidneys in 49 healthy students. As quantitative indicators of the excretory power of the kidneys, however, the two tests did not agree. The maximum urea concentration reading appears to be related to the quantity of urine in which it is excreted and perhaps, also, to the amount of urea normally being excreted by the individual.

#### COMPARISON OF RENAL FUNCTION TESTS IN 56 PATIENTS

In order to determine the relative value of the urea concentration test in the detection of disease, as well as to discover the importance of the observations just noted, 56 clinic and ward patients suspected of having some form of kidney disease were examined. Urinalysis, phenolsulphonephthalein and at least one urea concentration test were carried out on each and blood chemistry determinations were made on the majority.

The *phenolsulphonephthalein* test was carried out as in the case of the students but the dye was injected intramuscularly. A total excretion of 40 per cent of the dye or less was considered definitely abnormal while determinations ranging between this figure and 50 per cent were considered borderline.

cent) the generally accepted normal (25 per cent). Concentration of urea in the urine prior to the giving of urea varied from 0.6 per cent to 3.4 per cent with an average of 1.49 per cent. In Fig 1 the frequency distribution of these findings is shown, and the effect of the administration of urea on the urea concentration of the urine indicated.

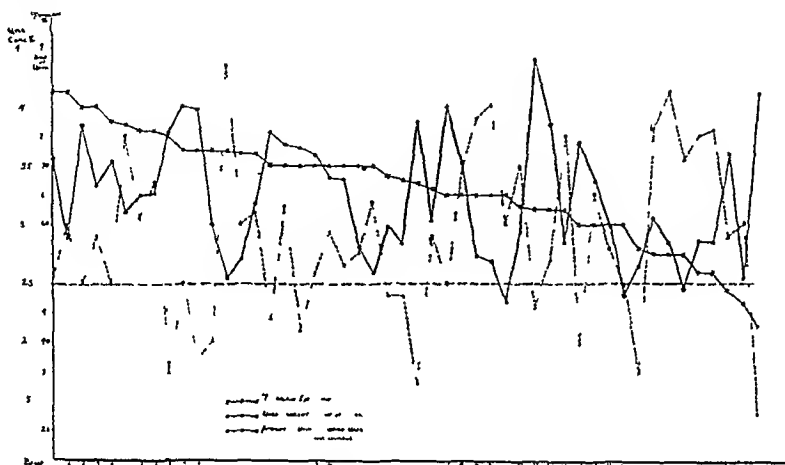


Chart I—Relation of phthalein excretion to maximum urea concentration of urine following ingestion of 15 gm of urea. Relation of amount of urine to urea concentration.

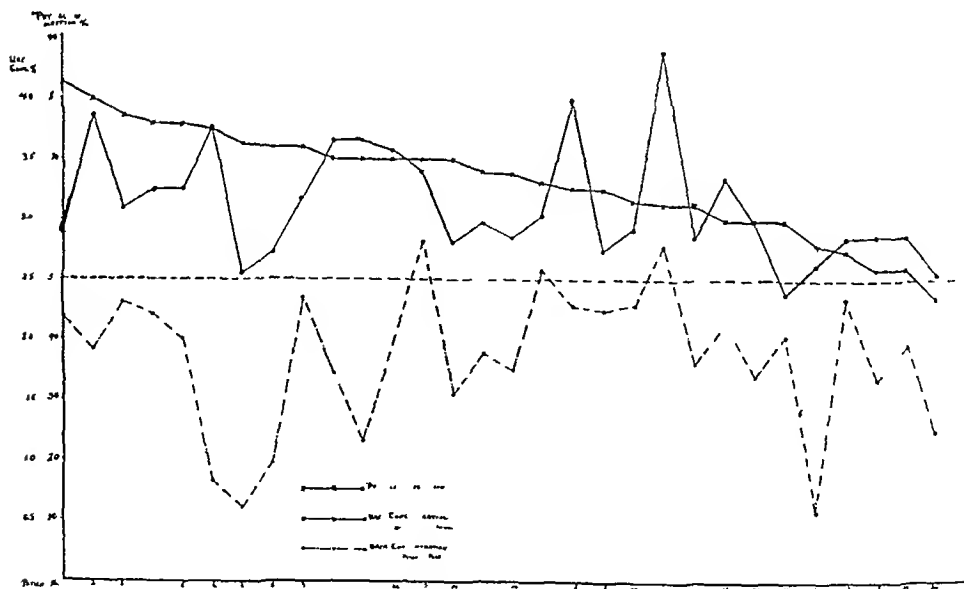


Chart II—Relation of urea concentration two hours following ingestion of 15 gm urea to urea concentration of urine prior to test.

Phenolsulphonephthalein excretion varied from 43 per cent to 82.5 per cent with an average of 66.3 per cent. Fig 2 shows the frequency distribution of these findings. It will be seen that the phenolsulphonephthalein reading was below 50 per cent in 3 instances (49, 47, 43 per cent, respectively).

Chart I is an attempt to determine the value of the urea concentration test as a quantitative indicator of renal function. The results of all the phenol sulphonephthalein determinations have been arranged in descending order, and, in this order, the phenolsulphonephthalein percentage and corresponding maximum urea concentration in each case are plotted. It is apparent that the two curves do not coincide as they should if they were both quantitative indicators of the excretory power of the kidneys.

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The phenolsulphonephthalein test was carried out as in the case of the students but the dye was injected intramuscularly. A total excretion of 40 per cent of the dye or less was considered definitely abnormal while determinations ranging between this figure and 50 per cent were considered borderline.

The *urea concentration* test was carried out as already described. The tests were made in the morning and the patients were allowed no food and little fluid after the preceding evening meal.

*Blood determinations* were made in the routine manner. Nonprotein nitrogen was considered normal up to 35 mg, urea nitrogen up to 15 mg, creatinine, 2.5 mg, and uric acid, 4.5 mg per 100 cc of blood.

Results of the examination of these 56 patients are given in detail in Table I and summarized in Table II.

#### ANALYSIS OF RESULTS

*Urinalysis*—Albumin or casts in the urine indicated the possible presence of renal disease in 60 per cent of the cases.

*Urea Concentration and Phenolsulphonephthalein Tests*—Concentration of urea was below normal in 60 per cent of the cases, while phenolsulphonephthalein was 40 per cent or less in 62.5 per cent of the cases and 45 per cent in 12.5 per cent. Of 17 cases of early or subacute nephritis (Table I, Groups 2 (b), and 4) the urea concentration test indicated the presence of disease in 16, while phenolsulphonephthalein excretion was abnormal in only 10. In other words, phenolsulphonephthalein excretion was normal in 7 patients who had other evidence of renal disease.

On the other hand, of 20 patients with predominating symptoms and signs of heart disease (Table I, Groups 2 (c), 2 (e), and 3) phenolsulphonephthalein excretion was 40 per cent or less in 15 and 45 per cent in 3 more, while urea concentration was below normal in only 9 individuals. Eleven patients with pronounced cardiac symptoms and much edema, but with little or no renal impairment, as indicated by normal urea concentration and negative urine and blood findings, showed a phenolsulphonephthalein excretion of 45 per cent or less. It appears that in many cases of cardiac disease with edema phenolsulphonephthalein excretion is a poor test of renal function, as in such cases the absorption of the dye by the peripheral circulation is greatly impaired. This is in harmony with the experience of Christian and O'Hare,<sup>7</sup> who state that in cardiac passive congestion phenolsulphonephthalein excretion may be moderately low without any real kidney disease.

*Nonprotein Nitrogen and Urea Nitrogen*—In only 23 per cent of the patients was there even slight abnormality of nonprotein nitrogen, while urea nitrogen was above normal in 43 per cent. This is consistent with the statement of other observers that an increase of the chemical constituents of the blood is a late sign of renal disease.

*Creatinine and Uric Acid*—Creatinine was above normal in only 1 patient, while uric acid was above 4.5 mg in 16 cases, 3 of these showed no other evidence of renal disease, while 4 had definite nephritis with normal nonprotein nitrogen and urea nitrogen.

Returning to the question of the value of the urea concentration test as a quantitative indicator of renal function it may be said that calculations devised to allow for the relation of amount of urine voided (Chart I) and concentration of urea prior to the test (Chart II) to maximum urea concentration have

TABLE I

## RENAL FUNCTION TESTS IN PATIENTS

NO	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG	PHTHALEIN PER CENT 130 MIN	UREA CONCENTRATION OF URINE										PLASMA
						BEFORE TEST		AFTER TAKING 1.0 GM UREA		2 HR AFTER						
						AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	
						1 Patients Showing no Evidence of Renal Disease										
1	21	124/84	0	12	50			08	2.24	06	3.07	Syphilis tested	Renal function			
2	20	120/80	0	12	50			90	1.18	52	2.52	Tonsillitis	Nocturia			
3	13		0	13	50			80	1.97	15	3.80					
4	20	119/80	0	13	45			96	2.31	3	3.45	Recovered from acute nephritis				
5	12	98/46	0	14	45			44	3.31	46	3.60	Constipation Syphilis Head				
6	20	120/92	0	14	40			80	1.98	27	3.59	Tonsillitis				
								82	2.32	95	2.97					
								92	1.45	61	3.44	Headaches				
2 Hypertension (a) With no Laboratory Evidence of Nephritis and no Signs of Heart Disease																
7	61	210/110	0	15	60			69	3.02	66	3.32	Nocturia ++				
8	49	226/120	0	14	85			78	2.44	28	3.00	G I Raging in L. ear				
9	37	186/84	0	13	80			60	3.04	74	3.45	Nocturia ++ Slight palp & dysp				
10	42	175/115	0	13	50			48	3.44	40	3.75	Focal infection Nocturia				
11	37	160/100	0	17	47			75	2.31	59	3.15	Headache Nocturia +				

Where other constituents of the blood are abnormal they are recorded under Remarks





TABLE I—CONT'D

NO	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG *	PITHALEIN PER CENT 130 MIN	UREA CONCENTRATION OF URINE										PELVIS	
						BEFORE TEST		AFTER TAKING 15 GM UREA									
						AMT URINE CC	PER CENT	1 HR AFTER		2 HR AFTER		AMT URINE CC	PER CENT	AMT URINE CC	PER CENT		
								AMT URINE CC	PER CENT	AMT URINE CC	PER CENT						
- Hypertension (d) With Well Marked Nephritis Few or no Cardiac Symptoms																	
1	32	104/108	Alb ++ Hy & GC ++ RBC +	60	10			120	151	112	170					Autopsy primary kidney dis- ease Died in uremia N P N 100 mg Uric acid 7 mg Advanced chr neph N P N 44 mg Uric acid 8 mg	
2	41	90/160	Alb +++ GC ++ RBC +	50	30	2.0	0.8	60	106	40	141					Nausea Edema Nocturia ++ N P N 70 mg Uric acid 6 mg Pellagra	
3	33	260/150	Hy & GC + RBC +	8	10			51	10	103	122						
4	62	140/100	Pus + Alb ++ Hy & GC + RBC +	10				90	11	8	191						
5	48	180/90	Pus + Alb ++	20				120	0.64	110	0.73					Dizziness Blinding spells Nocturia ++ Vertigo Dimness of vision Nocturia ++ N P N 40 mg Subacute neph with edema N P N 80 mg Uric acid 10 mg	
6	41	100/98	Pus + Alb ++ GC +	21	10	44	0.2	1	0.97	60	0.80						
7	36	108/138	Alb +++ Hy & GC + RBC +	40	10	92	0.38	24	0.78	39	0.79						
8	44	100/130	Pus + Alb +++ Hy & GC ++ RBC + Pus ++	20	40					100	1.42					Chr neph Heart failure con- trolled by digitals N P N 40 mg Uric acid 5 mg	

TABLE I—Continued

NO	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG %	HYPERTENSION 130 MIN	UPLA CONCENTRATION OF URINE										REMARKS
						BEFORE TEST		AFTER TAKING 15 CM		2 HR AFTER		UPLA				
						AMF URINE CC	1 HR AFTER AMT URINE CC	UREA PER CENT	AMT URINE CC	1 PEA PER CENT						
2 Hypertension (c) With Marked Heart Failure Predominating																
33	52	220/120	Alb ++	15	50		75	1.4	97		1.93	Fibrillation	Heart failure			
34	45	242/148	Alb ++ Hy C +	17	30		52	1.69	54		2.16	Marked heart failure	and 6 mg			
35	48	160/80	Hyb ++ Hy & GC + RBC + Pus ++	17	22		51	2.63	50		3.75	Heart failure				
36	59	190/140	Alb ++		40		98	1.29	90		1.79	Marked heart failure				
37	36	200/45	Alb ++		25		83	2.11	54		2.6	Aortic regurgitation with ful	ure			
38	15	208/150	0		20		98	1.66	85		1.99	Syphilitic heart disease with	failure			
39	52	160/58	Alb ++ Hy & GC + Pus ++	18	15		91	1.99	65		1.82	Heart failure	Ure acid 6 mg			
40	70	170/110	Alb + GC + Pus ++	19	15	115	100	1.02	104		1.10	Marked heart failure	N P N			
41	45	160/80	Pus +	15	20		92	0.93	99		1.04	Heart failure				
42	60	165/90	Alb + Hy & GC + Pus +		20		85	2.27	70		2.33	Syphilitic heart and	vascular			
43	49	155/115	Pus +++		38		50	3.00	66		2.98	Irregular and failing heart				
44	71	202/140	0		35	172	68	2.52	39		2.75	Nocturia +++				
45	32	170/120	Alb ++ GC + Pus +	15	25	80	34	3.14				Heart failure	Ure acid 6 mg			

TABLE I—CONT'D

NO	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG*	PHTHALEIN PER CENT 170 MIN	UREA CONCENTRATION OF URINE										REMARKS
						BEFORE TEST		1 HR AFTER		AFTER TAKING 15 GM UREA		5 HR AFTER		15 HR AFTER		
						AMT URINE CC	URFA PER CENT	AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	AMT URINE CC	UREA PER CENT	
						3 Heart Failure no Hypertension and no (?) Nephritis										
40	24	138/114	0	15	40	10	1.1	60	0.61	36	0.67	36	0.67	Acute heart failure Uric acid 0.5 mg		
41	36	124/88	Alb +	10	40	16	2.46	62	3.00	67	3.62	41	3.69	Aortic and myocardial heart disease St failure Uric acid 5.5 mg		
4 Subacute Nephritis With no Hypertension or Heart Disease																
42	24	100/100	Alb +++ Hv C +	90	5	100	0.54	51	0.66	36	0.67	36	0.67	Followed exposure to cold N P N 150 mg Creatinine 3.5 mg Uric acid 10 mg Followed streptococcal tonsillitis		
43	20	100/80	Alb ++ Hv & GC + RBC +++ Alb + Hv C +	10	60	60	1.38	102	1.64	50	1.37	50	1.37	Followed typhoid vaccination		
44	18	130/90	Alb +++ Hv & GC ++	14	20	70	0.4	91	1.07	60	1.7	60	1.7	Caused Uric acid 0.5 mg		
45	31	130/90	Alb +++ Hv & GC ++	14	40	96	1.51	96	1.51	60	2.19	60	2.19	Uric acid 0.5 mg		
46	5	140/94	RBC + Alb ++ Hv & GC +	14	30	50	1.33	22	1.38	32	2.09	32	2.09	Uric acid 0.5 mg		
47	13	110/70	RBC + Alb ++	14	30	100	1.60	100	1.60	70	2.42	70	2.42	Followed tonsillitis		
48	41	100/80	GC ++	14	40	100	1.51	100	1.51	34	2.31	34	2.31	Mercurial		
49	64	120/70	Alb + Hv C +	14	30	100	1.51	100	1.51	44	2.08	44	2.08	Bronchiectasis		
50	44	110/69	RBC & Pus ++ Hv & GC +	19	60	18	1.78	25	2.34	64	2.10	64	2.10	Focal nephritis		

TABLE II  
SUMMARY OF RENAL FUNCTION TESTS IN 36 PATIENTS

CASES		URINALYSIS SHOWED ABNORMALITY IN	BLOOD				PTHIALEIN EXCRETION		MAXIMUM UREA CON- CENTRATION BELOW 25 PER CENT IN	SYMPTOMS SUGGESTIVE OF NEPH- RITIS IN
	NO		NON-PRO- TEIN NITROGEN ABOVE 35 MG IN	UREA NITROGEN ABOVE 15 MG IN	(CIPUTININE ABOVE 2.5 MC IN	URIC ACID ABOVE 4.5 MG IN	40 PER CENT OR LESS IN	ABOVE 40 PER CENT BELOW 50 PER CENT IN		
1	No evidence of renal disease	0	0	0	0	0	1	2	0	2
2	Hypertension (a) with no nephritis (b) with beginning signs of nephritis but few or no cardiac symptoms	5	0	1	0	0	0	1	0	1
	(c) with beginning cardiac disease, little or no nephritis	8	0	1	0	0	4	0	7	3
	(d) with well marked nephritis, few or no cardiac symptoms	5	4	4	0	2	3	2	1	0
	(e) with marked heart failure pre-dominating	8	6	6	0	3	8	0	8	8
3	Heart failure	13	1	4	0	3	12	0	8	
4	no hypertension, no (f) nephritis	2	0	0	0	2	1	1	0	1?
	Subacute nephritis									
	no hypertension or heart disease	9	2	3	1	3	6	1	9	9
	Total	36	13	21	1	18	35	7	3	29

failed to improve the significance of the test. The most obvious solution to the hypothetical failure of the urea concentration test as a quantitative indicator of renal function, considered in the discussion of Charts I and II is the calculation of the total amount of urea excreted in two hours. This was done in 49 students and found to range from 2.2 gm. to 5.2 gm. with an average of 3.9 gm. Patients suffering from various grades of nephritis excreted from 0.5 gm. to 3.8 gm. In cases of severe renal damage, total urea excretion was very low, but in moderate degrees of nephritis total urea excretion often greatly exceeded the lower limits of normal observed in students. It appears, then, that it is the concentrating power of the kidney, rather than the amount of urea excreted, that is first affected in nephritis.

A true conception of the value of the urea concentration test as a quantitative indicator of renal function can only be gained by following the progress of a large number of cases with repeated tests. As this was done in only a relatively few cases, no more can here be said than that the urea concentration test showed some relation to the degree of renal impairment. Jones and Cantarow<sup>9</sup> suggest that the percentage variation between urea concentration prior to the test and urea concentration following ingestion of urea is more significant of renal damage than maximum urea concentration alone. Judging by the data for the 56 patients here reported, however, little additional information is to be gained by the detailed calculation of percentage variation.

*Conclusions*—Power of concentration appears to be a definite function of the kidney and one which is early involved in the damage produced by nephritis. Thus, most observers agree that the earliest sign of kidney damage as shown by laboratory tests is an increase of the volume of the night urine and a decrease in its specific gravity or, in other words, a decrease in its concentration. MacLean's test measures the concentrating power of the kidney in response to a definite stimulus by a method which is even more simple than that necessary for the carrying out of the various water concentration tests, and in both theory and practice is a valuable test of renal function. Where there is congestive cardiac failure, the urea concentration test probably gives a more correct conception of the functional state of the kidneys than does the phenolsulphonephthalein test. As Rabinowitch points out<sup>10</sup> no single test gives a complete and uniform idea of renal function and various types of nephritis show varied responses to each test. The urea concentration test is as important in the detection of renal disease as is the widely used phenolsulphonephthalein test, and because of its simplicity is particularly valuable for the physician with few laboratory facilities.

#### SUMMARY

1 Urea concentration and phenolsulphonephthalein tests in 49 healthy individuals are compared and the value of these tests as well as of urinalyses and blood chemistry determinations is considered in 56 patients.

2 The nature of the urea concentration test and the relation of maximum urea concentration to total urine volume and to urea concentration of urine prior to the ingestion of urea are examined.

## REFERENCES

- 1 MacLean, H., and de Wesselow, O. L. V. *Brit J Exper Path* 1 53, 1920
- 2 Rabinowitch, I. M. *Arch Int Med* 32 927, 1921
- 3 Harrison, G. H. *Brit J Exper Path* 3 28, 1922
- 4 Rabinowitch, I. M. *Canad M A J* 16 492, 1926
- 5 MacLean, Hugh. *Modern Methods in the Diagnosis and Treatment of Renal Diseases*  
Lea and Febiger, Philadelphia, 1927, p. 59
- 6 Bowen, B. D. *Am J M Sc* 174 769, 1927
- 7 Christian, Henry A., and O'Hare, James P. *Oxford Medicine*, Oxford University  
Press, New York. Supplemented to 1926, 3 630
- 8 Wintrobe, M. M. *Proc Soc Exper Biol & Med* 26 40, 1928
- 9 Jones, H. W., and Cantarow, A. *Arch Int Med* 38 581, 1926
- 10 Rabinowitch, I. M. *Arch Int Med* 28 827, 1921

## AMINO NITROGEN DETERMINATION IN BACTERIOLOGIC MEDIA\*

By D. A. MACPIERSON AND F. P. BROOKS,† CHAPEL HILL, N. C.

IN RECENT years the bacteriologist has resorted to minute biochemical analyses in an attempt to facilitate the differentiation of closely allied strains. The determination of amino nitrogen has become a common procedure in the study of protein metabolism by the bacteria.

Sorensen's formol titration,<sup>1</sup> Van Slyke's nitrous acid method,<sup>2</sup> and the newer colorimetric method of Fohn<sup>3</sup> have all been employed. Because of its speed and reported accuracy the van Slyke method has been most frequently chosen recently.

Several investigators have reported unsatisfactory results with the Van Slyke apparatus as applied to bacteriologic culture media. The difficulty has been in obtaining consistent results on duplicate samples of the same media.

As has been pointed out by Lamson,<sup>4</sup> Sturges and Rettger, and Wagner, Dozier, and Meyer,<sup>5</sup> found it necessary to make several determinations by this method before satisfactory checks could be obtained. Lamson<sup>4</sup> concluded that Van Slyke's method yields very irregular results, and is unsatisfactory for solutions low in amino nitrogen. De Bord<sup>7</sup> abandoned the method upon finding as much as 18 per cent variation in a series of tests on sterile peptone solution.

In the course of an investigation on the enzymes of the streptococci by one of us, the Van Slyke method was adopted for the determination of protein cleavage. Despite great care in attention to all details of the procedure, satisfactory checks could not be obtained. One example will suffice to illustrate the difficulty.

The medium tested was sterile veal infusion broth adjusted to a  $P_H$  7.6. The Van Slyke micro apparatus was employed, using a 2 c.c. sample and Pfanstiel's caprylic alcohol for the prevention of foaming. Table I indicates

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the results in one experiment. The amount of gas from the blank on the reagents has been deducted. Temperature and pressure remained constant. Ammonia was not removed.

The results in Table I, representative of many other such experiments, indicate the inconsistency we experienced with this method. A maximum difference of 44.9 per cent between checks made it quite obvious that the method could not be used. It seemed to us that with a method commonly relied upon by bacteriologists it would be worth while to determine the reason for such inconsistency. We desire, therefore, to report our findings regarding the reasons for the inconsistent results by the Van Slyke method, and a comparison of this method with those of Sorensen and Folin as applied to bacteriologic media.

TABLE I  
VAN SLYKE NITROGEN ( $\text{NH}_2 + \text{NH}$ ) IN STERILE VEAL INFUSION BROTH

SAMPLE	GAS IN CC	AMINO N ( $+\text{NH}_2$ ) MG PER 100 CC	PER CENT DEVIATION FROM THE MEAN
1	187	50.55	+26.3
2	103	28.00	-30.0
3	139	37.78	-5.5
4	121	32.89	-17.7
5	168	50.83	+27.0
Mean	144	40.00	

Upon examination of the literature we found a reference to the importance of the caprylic alcohol foam depressant in a paper by Bock.<sup>4</sup> In a footnote he calls attention to the necessity for purifying the caprylic alcohol.

An experiment was undertaken to determine the relation of the caprylic alcohol to the discrepancy in our results. Some of the Pfanstiehl caprylic alcohol, which we had been using, was purified according to the method of Bock. Since this process of purification has not been brought sufficiently to the attention of bacteriologists we quote the procedure here. The alcohol is shaken twice (best in a separatory funnel) with a mixture of glacial acetic acid and  $\text{NaNO}_2$  solution (30 gm in 100 cc  $\text{H}_2\text{O}$ ) the acid and the nitrate being in the proportion of 1:5. The alcohol is then washed with a little water two or three times, transferred to a distilling flask, a very small fraction of  $\text{NaOH}$  added and distilled under reduced pressure.

Table II shows the results of determinations with this purified Pfanstiehl caprylic alcohol as compared with the unpurified Pfanstiehl and several other brands. The determinations were made using the regular reagents, 2 cc

TABLE II  
VOLUME OF GAS PER CC CAPRYLIC ALCOHOL, 0 C., 760 MM PRESSURE

PURIFIED		UNPURIFIED			
SAMPLE	PFANSTIEHL	PFANSTIEHL	MERCK'S	COLEMAN BELL	EASTMAN
1	0.1615 cc	4.69 cc	0.50 cc	0.104 cc	0.630 cc
2	0.175 cc	4.44 cc	0.56 cc	0.076 cc	0.396 cc
3	0.166 cc	4.52 cc	0.220 cc	0.114 cc	0.622 cc
4			0.230 cc	0.034 cc	0.604 cc
5				0.090 cc	
Average	0.1677 cc	4.5 cc	0.27 cc	0.094 cc	0.613 cc



distilled water as the sample, and 0.5 cc of the caprylic alcohol in each case. The figures indicate the volume of gas derived per cc of alcohol, calculated to 0° C and 760 mm pressure.

From Table II it is evident that there is a wide variation in the purity of commercial caprylic alcohols. The sample used should be purified if preliminary tests indicate appreciable variation or large amounts of gas.

The Bock<sup>s</sup> method of purification yields an alcohol which gives consistently satisfactory checks on blank determinations. It is further evident that in the Van Slyke procedure the amount of caprylic alcohol must be accurately measured.

The effect of the shaking period on the volume of gas obtained from the purified caprylic alcohol was next determined. The usual reagents were used plus 2 cc of water as the test material, with and without the purified alcohol. Three minutes were allowed for absorption, and the shaking interval was varied. Table III indicates the results.

TABLE III  
EFFECT OF SHAKING TIME ON VOLUME OF GAS IN BLANK DETERMINATIONS

SAMPLE	REAGENTS PLUS 2 CC H <sub>2</sub> O, NO ALCOHOL	REAGENTS PLUS 2 CC H <sub>2</sub> O, 0.2 CC PURIFIED ALCOHOL	
	SHAKEN 5 MIN	SHAKEN 5 MIN	SHAKEN 10 MIN
1	0.09 cc	0.27 cc	0.41 cc
2	0.11 cc	0.30 cc	0.40 cc
3	0.13 cc	0.26 cc	0.39 cc
4	0.13 cc		
5	0.10 cc		
6	0.11 cc		
Average	0.11 cc	0.28 cc	0.40 cc

From the above experiment it can be seen that a definite shaking period must be used in the test, and that it should be the same as that employed in the blank on the reagents. It is also apparent that even the purified caprylic alcohol adds an important amount of gas to the total volume.

Having established the fact that satisfactory checks could be obtained with the purified alcohol on blank determinations, we again applied the method to veal infusion broth. Table IV shows the results obtained on two different lots of sterile veal infusion broth adjusted to  $P_H$  7.6. Two cc of the

TABLE IV  
VAN SLYKE AMINO NITROGEN IN STERILE VEAL INFUSION BROTH

SAMPLE	BROTH NO 1		BROTH NO 2	
	MG PER 100 CC	PER CENT DEVIATION FROM MEAN	MG PER 100 CC	PER CENT DEVIATION FROM MEAN
1	36.55	-7.7	39.20	+0.9
2	38.50	-2.7	38.85	0.0
3	41.00	+3.5	38.20	-1.6
4	40.50	+2.2	38.45	-1.0
5	41.50	+4.7	39.35	+1.3
6			38.05	-2.0
7			39.50	+1.6
8			39.20	+0.9
Mean	39.60		38.85	

test sample and 0.2 c.c. purified caprylic alcohol were used, allowing a five minute shaking period followed by three minutes absorption. The free  $\text{NH}_3$  was determined by the Folin<sup>9</sup> aciation method, and was found to be 10 mg per 100 c.c. of broth. This amount has been deducted from the results in Table IV.

The data in Table IV represent the unselected results in successive determinations. It can be seen that these results are fairly consistent, and within the experimental error in measuring such small quantities of gas.

#### A COMPARATIVE STUDY OF THE VAN SLIKE AND FOLIN METHODS FOR THE DETERMINATION OF THE AMINO NITROGEN IN CULTURE MEDIA

While the data reported above satisfied us that the Van Slyke method would give satisfactory results with certain precautions we thought it advisable to compare this method with the newer colorimetric one of Folin.<sup>9</sup>

Two samples of sterile 1 per cent Difco peptone water were examined by these two methods. One was unadjusted, and the other buffered with phosphates to a  $\text{pH}$  7.6. Table VI indicates the results obtained. Ammonia was not removed, since Nessler's reagent failed to show any  $\text{NH}_3$  present.

TABLE VI

AMINO NITROGEN (PLUS  $\text{NH}_3$  N) IN 1 PER CENT PEPTONE WATER BY VAN SLIKE'S AND FOLIN'S METHODS

SAMPLE	MG PER 100 CC			
	UNBUFFERED		BUFFERED	
	VAN SLIKE	FOLIN	VAN SLIKE	FOLIN
1	6.45	4.74	5.40	4.68
2	5.95	4.78	5.50	4.74
3	5.65	4.65	5.20	4.60
4			5.40	
5			5.20	
Mean	6.01	4.72	5.35	4.60

An average difference between the methods of 21.4 per cent for the unbuffered and 12.6 per cent for the buffered peptone water was larger than we anticipated. The lack of agreement may be explained by the difficulty in measuring such small quantities of gas as were obtained from 2 c.c. samples of a medium low in amino nitrogen.

In order to check the accuracy of the methods a definite known amount of amino acid, giving a larger volume of gas, was added to the peptone water media. Standard glycine solutions were prepared by adding 25 mg of nitrogen in the form of glycine to 50 c.c. portions of the buffered and unbuffered peptone water. Using the value 4.72 mg per 100 c.c. peptone water and 4.69 mg per 100 c.c. unbuffered peptone water from Table VI as the amino nitrogen content of the respective media, the addition of 5 c.c. of standard glycine solution (representing 5 mg amino N) to 100 c.c. of the media would give a calculated amino nitrogen content of 9.25 mg and 9.23 mg respectively. The amount of amino nitrogen actually determined experimentally by both methods is indicated in Table VII. Nessler's reagent showed no  $\text{NH}_3$  present.

With the added amount of amino nitrogen the results are very acceptable particularly by the Van Slyke method. This would indicate that the error with the Van Slyke method in Table VI is largely due to the small amount of gas measured.

The two methods were now applied to the determination of amino nitrogen in two lots of veal infusion broth. The results of this comparison are indicated in Table VIII.

TABLE VII

DETERMINATION OF KNOWN AMOUNTS OF AMINO NITROGEN ADDED TO PEPTONE WATER

SAMPLE	MG PER 100 C C			
	UNBUFFERED PEPTONE PLUS GLYCINE		BUFFERED PEPTONE PLUS GLYCINE	
	VAN SLYKE	FOLIN	VAN SLYKE	FOLIN
1	9.20	8.60	9.15	9.15
2	9.75	7.85	9.30	9.15
3	8.95	8.70	9.30	8.75
Average	9.30	8.65	9.24	9.02
Theoretical	9.25		9.23	

TABLE VIII

NH-NH<sub>2</sub> NITROGEN IN VEAL INFUSION BROTH MG PER 100 C C

SAMPLE	BROTH NO 1		BROTH NO 2	
	VAN SLYKE SHAKEN 5 MIN	FOLIN	VAN SLYKE SHAKEN 10 MIN	FOLIN
1	34.60	41.30		
2	36.00	40.25	60.20	59.7
3	36.20	40.50	60.30	58.8
4	37.30			
5	44.15*			

\*Shaken ten minutes

The results in Table VIII fail to show as satisfactory agreement between the methods as was obtained with the peptone water media. The broth was not freed from ammonia, but since this reacts with the reagents of both methods, it does not vitiate the results so far as a comparison is concerned. As is indicated by Sample No. 5 of Broth No. 1 and the results with Broth No. 2, a ten-minute shaking period in the Van Slyke method yields a larger amount of amino nitrogen. Furthermore, this amount agrees better with the results obtained by Folin's method. If one wishes the actual amount of amino nitrogen present it is necessary to determine a sufficient reaction time for the particular medium. For a simple comparison of proteolysis at various time intervals, an arbitrary standard shaking period may be sufficient.

The two methods were now applied to the determination of amino nitrogen in veal infusion broth cultures of hemolytic streptococci. One-half cubic centimeter of an eighteen-hour veal infusion broth culture was added to each of two flasks containing 50 c c sterile veal infusion broth. After twenty-four and forty-eight hours' incubation at 37° C, a flask was removed for amino nitrogen and ammonia determinations. Stained smears established purity and growth. In order to ascertain the effect upon the two methods of not removing the ammonia, determinations were made before and after the ammonia

was removed. Ammonia was determined by the aeration method of Folin.<sup>10</sup> The amino nitrogen determinations after removal of free ammonia were made on samples following the aeration process. They, therefore, were saturated with sodium chloride. Table IX shows the collected data.

As will be seen the results by the two methods compare well with one another. So far as comparable results go, the presence of free ammonia has little effect. It reacts equally with both methods. If one adds together the  $\text{NH}$  nitrogen obtained after removal of the ammonia and the  $\text{NH}_3$  determined by the aeration process, the result compares favorably with the  $\text{NH}_3$  nitrogen obtained by either method before removal of the ammonia. The theoretical error introduced by utilizing the sample from the aeration process for the determination of  $\text{NH}$  nitrogen is negligible.

TABLE IX

$\text{NH}-\text{NH}_3$  NITROGEN CONTENT OF BROTH CULTURES BEFORE AND AFTER REMOVAL OF AMMONIA  
MG PER 100 CC

MATERIAL	BEFORE REMOVAL OF $\text{NH}_3$					
	24 HR INCUBATION			48 HR INCUBATION		
	VAN SLYKE $\text{NH}-\text{NH}_3$	FOLIN $\text{NH}-\text{NH}_3$	$\text{NH}_3$ AERATION	VAN SLYKE $\text{NH}-\text{NH}_3$	FOLIN $\text{NH}_3$	$\text{NH}_3$ AERATION
Control unincubated	57.0	50.0	8.9	57.2	46.8	8.5
Control incub 37° C	56.0	51.7	8.9	53.0	48.4	8.1
Culture Hemo No 2	61	66.6	15.0	63.0	69.0	14.9
Culture No 827	67.2	67.0	15.5	63.5	68	15.0

MATERIAL	AFTER REMOVAL OF $\text{NH}_3$			
	24 HR INCUBATION		48 HR INCUBATION	
	VAN SLYKE $\text{NH}$	FOLIN $\text{NH}$	VAN SLYKE $\text{NH}$	FOLIN $\text{NH}$
Control unincubated		---	45.0	42.2
Control incub 37° C	41.7	---		41.7
Culture Hemo No 2	52.7	---	55.5	51.5
Culture No 827	47.0		32	3

COMPARISON OF THE VAN SLYKE, FOLIN AND SORESENSEN METHODS FOR AMINO  
NITROGEN DETERMINATION IN BACTERIOLOGIC MEDIA

Since the Sorensen method<sup>1</sup> for the determination of amino nitrogen has been extensively used by bacteriologists in previous years, we believed it worth while to compare it with the two newer methods. In order that the formal titration should compare as favorably as possible with the other two methods, we adopted for this titration the newer technique of Brown's<sup>11</sup> Method 'B'.

The unselected successive results with the three methods on veal infusion broth and on 1 per cent peptone water are shown in Tables X and XI. Ammonia was removed from the veal infusion by treatment with permuted lime. The peptone water was buffered to pH 7.6 with phosphates. Nessler's reagent failed to show appreciable ammonia in this medium.

Tables X and XI show a wide variation in the results of the three methods. The Folin method shows 16.8 per cent less amino nitrogen than the Van Slyke, while the Sorensen results are 9.3 per cent below those of the Folin method. However, the maximum deviation from the mean of the individual

samples in the Van Slyke (21 per cent) and Folin (28 per cent) methods is acceptable. The Sorensen results show a greater deviation (99 per cent) from the average.

Comparison between the methods on the buffered peptone water is even less satisfactory. Here there is an average difference of 24.5 per cent between the Van Slyke and Folin methods. The Sorensen results are 14.9 per cent lower than those of the Folin method. The maximum variation of the individual samples from the average is greater (79 per cent) here for the Van Slyke, but somewhat less (23 per cent) for the Folin method. The deviation from the average in Sorensen's method is greater (182 per cent) than appeared with the veal infusion. The presence of phosphates is known to affect the formol titration, but Brown's technic supposedly overcomes this difficulty.

TABLE X  
AMINO NITROGEN IN STERILE VEAL INFUSION BROTH

SAMPLE	VAN SLYKE	FOLIN	SORENSEN
	MG PER 100 CC	MG PER 100 CC	MG PER 100 CC
1	49.6	42.20	42.0
2	50.5	42.10	38.5
3	51.5	41.85	38.5
4	50.5	42.55	35.0
5	51.5	40.95	37.0
6	50.4	42.90	
Mean	50.7	42.15	38.2

TABLE XI  
AMINO NITROGEN IN STERILE BUFFERED PEPTONE WATER

SAMPLE	VAN SLYKE	FOLIN	SORENSEN
	MG PER 100 CC	MG PER 100 CC	MG PER 100 CC
1	34.55	24.63	17.5
2	33.60	25.60	21.0
3	30.80	25.13	18.9
4	33.15	24.84	23.8
5	36.00	25.75	24.5
6	32.00	25.00	21.0
7	---	---	23.1
Mean	33.35	25.15	21.4

We are unable to explain the variation between the methods on the same medium. The Folin method is, of course, based upon a comparison between a known standard amino acid solution and the unknown. The standard we used was the glycine solution recommended by Folin, and the sole guarantee of its accuracy rests upon the weighing of the glycine. This was done with great care on an analytical balance. In order to check this standard amino acid solution to some degree, we determined the amino nitrogen content of the standard glycine solution by both the Van Slyke and Sorensen methods.

The theoretical content of the glycine solution was 100 mg of amino nitrogen per 100 cc of the solution. The average result by Van Slyke's method was 105 mg, while the Sorensen titration showed 98.2 mg. If the standard glycine solution used actually contained 105 mg of amino nitrogen, then the Folin results should be increased. The Folin and Van Slyke results would then be more comparable, but the divergence between the Folin and

Sorensen results would be greater. On the other hand the Folin method considers the careful weighing of the glycine as accurate, and glycine is not well determined by the Van Slyke method. We prefer, therefore, to consider the results as they stand.

#### DISCUSSION

From the above tabulated data it is not possible to decide which of the three methods determines the content of amino nitrogen most accurately. As a general rule, however, the bacteriologist is more interested in variations in the amino nitrogen over a certain period of time than he is in the absolute amount present. For this purpose probably any one of the three methods could be used.

The Van Slyke method has been popular because of its speed. We believe that where only a few determinations are to be run and the results must be known as soon as possible, that this method is the one of choice. Certain precautions and attentions to detail are necessary in order that acceptable consecutive results may be obtained. A definite uniform amount of purified caprylic alcohol must be used throughout the determinations. So also a definite shaking speed and shaking period must be adhered to throughout. The same sample will not show the same amount of amino nitrogen if shaken for three minutes and again ten minutes nor will it show the same amount if the speed of shaking is once fast and then slow.

What has been said applies to both the blank on the reagents and the actual determinations. They must all be manipulated in exactly the same manner. It is to be remembered that the actual amount of nitrogen gas measured is very small, and that a slight error in measurement will be magnified greatly in the computations. Under these conditions the method has given acceptable successive results in our hands. We are unable to explain why DeBord<sup>7</sup> and Lamson<sup>8</sup> failed to get satisfactory checks in the main tests when they used a purified alcohol and obtained consistent checks in the blank determinations.

Where a large number of determinations are to be made and one can wait until the next day for the results the Folin method is to be preferred. Only one determination can be made at a time with the Van Slyke apparatus while with the Folin method a large series may be set up and the reagents pipetted for all tubes at one time. A large series then, may be determined in less time than with the Van Slyke procedure even though they must sit overnight before reading.

This method has the advantage of employing the colorimeter which is widely available and which can be used for other purposes than the determination of amino nitrogen. The Van Slyke apparatus is very fragile, and is apt to be broken with the press of work causing considerable inconvenience. The apparatus cannot of course be used for any other purpose. Our results indicate that the Folin method gives more uniform results than the Van Slyke. There appears to be less difference between consecutive results (about 4 per cent) and less variation from the average (about 3 per cent).

The Sorensen method requires accurate titrations between very accurate

$P_H$  limits Some speed in the determination is necessary to prevent loss of  $CO_2$ . It is a longer, more involved method than the other two, and our results appear less satisfactory with it. The maximum variation (about 22 per cent) between consecutive results, and the deviation from the average (about 14 per cent) are greater than with either one of the other methods.

#### SUMMARY

1 With certain precautions, particularly the use of a definite amount of purified caprylic alcohol, the Van Slyke method for the determination of amino nitrogen values gives acceptably consistent results on peptone water and veal infusion broth.

2 A comparison of the Van Slyke, Folin and Sorensen methods for amino nitrogen determination indicates that the Folin method is the most consistent, and is best suited for a large number of determinations.

#### REFERENCES

- 1 Henriques, V, and Sorensen, S P L. *Biochem Ztschr* 7 45, 1907, *Ztschr f phys Chem* 63 27, 1909
- 2 Van Slyke, D D. *J Biol Chem* 9 185, 1911, 12 275, 1912, 16 121, 1913, 23 407, 1915
- 3 Folin, O. *J Biol Chem* 51 377, 1922
- 4 Lamson, R W. *J Bact* 9 307, 1924
- 5 Sturges, W S, and Rettger, L F. *J Bact* 7 551, 1922
- 6 Wagner, E, Dozier, C, and Meyer, K. *J Infect Dis* 34 63, 1924
- 7 De Bord, G. *J Bact* 8 7, 1923
- 8 Bock, J C. *J Biol Chem* 28 357, 1916 17
- 9 Folin, O, and Macallum, A B. *J Biol Chem* 11 523, 1912
- 10 Folin, O. *Ztschr f phys Chem* 37 161, 1902 3
- 11 Brown, J H. *J Bact* 8 245, 1923

### COLORED GLASS STANDARDS\*

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#### I. BLOOD SUGAR

THE use of colored glass standards in place of variable solutions is much desired for the small laboratory. The securing of correct standard solutions by those whose training or equipment does not permit the making of them is a very real problem, doubly so where the solutions do not keep for any length of time. In the hope of performing some such service, particularly for our own students, we have cooperated with the Klett Manufacturing Company in the preparation of glass standards.

The standard most desired is that for blood sugar, and the one which has given us the most trouble. We followed Folin's new method<sup>1</sup> and found that scarcely two standards prepared from the same known glucose solution gave exactly the same color. We found it necessary, if we wished to get anything like the same shade of blue, to duplicate our procedure exactly. Calibrated apparatus, of course, was used throughout.

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Immediately after introducing the glucose solution and alkaline copper tartrate into the sugar tube, our procedure was to heat it in the boiling bath for exactly six minutes, to transfer it to a cold water bath for exactly four minutes, to add the molybdate, dilute, and read. If the solution is diluted at once the color fades rapidly. In general, the longer the molybdate mixture stands before dilution, the more blue the diluted solution and the longer it takes to fade. Thus Dr. Katayama had observed in the routine analysis of our blood sugars. Since he found that with twenty or thirty tubes the fading in color was so pronounced as to prevent proper matching of colors, he regularly waited fifteen minutes before diluting. Our waiting periods ranged from no minutes to three days. A glass, that matched perfectly 15 mm. of solution which had been allowed to stand twenty minutes before diluting appeared very gray and purplish against the deep bright blue of a solution that had stood three days after the addition of the molybdate. The latter solution underwent no observable fading for twenty-four hours after diluting. Standards diluted in less than twenty minutes were green and gray against the glass, those later than twenty minutes were too blue.

In the hundreds of glucose solutions prepared, not one was found that within the five minute period immediately following dilution ever gave consistent or certain readings. The color is exceedingly variable. At the beginning there is more red in the color, this rapidly disappears. This shift in colors is followed by a period, usually of not more than five minutes, during which the color is stable. Then the blue fades, leaving green and gray to predominate.

This variation in color is of more than theoretic interest. If a blood such as an emergency specimen is brought in for analysis when the routine specimens have just received the molybdate, is it permissible to hold them at that stage, to run the new specimen through and to match it with the others? It is very convenient, it is economical both of time and of reagents. We did just this. The same filtrate was used and the analyses made simultaneously. The one matched against the standard that had waited gave a sugar of 0.16 per cent. The other matched against a fresh standard prepared with it gave 0.25 per cent.

The rate of fading after dilution is important in determining how soon the colors must be matched. To take a single instance, the unknown reads 10.6 against both the glass standard and the solution standard set at 15 mm. for twelve minutes. Thirty minutes after diluting the unknown reads 8.7 to 8.9 against the standard set at 15. Against the glass it reads 13. Depending on when and against what the reading is taken, the sugar in the blood would be read as 0.141, 0.170, or 0.115 per cent.

Because of the uncertainty of our results with Folin's modification, we have returned to the old Folin-Wu method.

This method, while not showing the extreme variations encountered with the new Folin reagents, is subject to the same limitations.

With the glass now available for use, the unknowns must be boiled vigorously six minutes and cooled for three minutes. After the addition of molyb-



date, fifteen minutes must elapse before diluting, and then another five minutes before reading. Color comparisons must be made within the following ten minutes.

The blue color developed depends upon many unknown factors. Where the glass standard is used the limitations are more obvious than with solutions. Since it is possible to produce an almost infinite variety of blue colors with *standard glucose solutions*, the glass standard can give accurate results only when the directions are obeyed explicitly.

Wherever the glass disc is used, *each* worker should determine his own corrective factor. Working conditions, such as, light and temperature, quality and age of reagents used, and personal variations in color comparisons will all affect the factor. Nevertheless, we venture to say that there is probably much less error in using a standardized glass, together with the factor determined by the maker, than in using standard solutions bought from ordinary commercial houses or prepared by the average technician.

The chemicals employed in preparing the reagents used may vary the factor tremendously. The copper reagent should be a clear deep blue. Whenever much sediment appears or the blue color seems faded, poor results will be obtained. When our molybdate is fresh (at least for ten to fourteen days after preparation), it gives less blue with cuprous oxide than when it is a month old.

In attempting to standardize the glass we had repeated setbacks. Just as we were about to draw conclusions, all our known solutions would suddenly shift their colorimetric reading 1 to 2 mm higher than they had been running. Finally we discovered that this difference was evident every time new reagents were prepared. The new molybdate was found to be the troublesome factor. At present we recommend that the phospho molybdate reagent be prepared two or three weeks before use. This solution, however, will stand further investigation before any decision can be given as final. Wu has already observed that the complex phospho molybdic-tungstic acids give different colors or different shades on slight variations in conditions.<sup>3</sup> Since these complex acids may be easily reduced, giving a good blue color, they have been regarded as admirable color reagents. They are, but not in the hands of a careless or ignorant technician.

It is impractical to publish pages upon pages of figures to show exactly why the molybdate is allowed fifteen minutes before dilution. We do not claim that it is even ideal, but it is the shortest period that we have been able to find that resulted in a color stable enough to permit the reading of several unknowns. The actual number of determinations that can be run at once depends upon the speed of the worker in color matching.

The temperature of the cold water-bath affects the final color. Duplicates were run, some being cooled in water kept at 20 to 24° C and the others in water containing ice. It was found that those cooled at 20° C gave an excellent match with the glass disc. Those cooled in ice water were such a clear green-blue that the glass seemed cloudy and distinctly purplish against them.



Unless the worker prepares his own series of corrective factors, the color comparisons should be confined to a period of five to ten minutes following the five minutes of waiting for a stable color. The factor as determined by us for this period is 1.2 (multiply the colorimeter reading by this and apply the corrected figure to the usual calculation). If conditions are just right, it is possible to make decent comparisons for as much as twenty minutes after dilutions, but no one corrective factor can be applied to such a series of readings. The glass is used without either cup or plunger.

Table I shows the sort of results that we have invariably obtained when reading unknowns against both glass and standard solution. That we could get such excellent checks was a great surprise to us since we believed before we started that our figures would demonstrate that the glass disc could not be used in routine work.

Table II shows the typical shift in color seen in the standard glucose solution.

TABLE II  
CORRECTIVE FACTOR FOR SUGAR DISC

TIME AFTER DILUTING	READING OF STANDARD SOLUTION*	REMARKS	CORRECTIVE FACTOR
5 minutes	12.3	Fair match	
6 minutes	12.4		1.21
7 minutes	12.5	Excellent match	1.20
10 minutes	12.7		1.18
12 minutes	12.9		1.16
14 minutes	13.0	Harder to match	1.15
15 minutes	13.1		1.14
20 minutes	13.4	Gray	1.12
25 minutes	—	Too gray to match	

\*The worker must allow for the fact that bubbles of CO<sub>2</sub> may collect on the plunger otherwise that will cause a shade to appear. Between readings the plunger should be raised above the level of the fluid in the cup.

Our standard glucose solution is prepared with saturated benzoic acid. This acid affects the depth of color produced. Three standards were prepared: (1) One hundredth per cent glucose in distilled water, (2) one hundredth per cent glucose in half saturated benzoic acid, and (3) the same in fully saturated benzoic acid. With (1) set at 15, (2) read 13.4, and (3) read 12.4. This experiment was repeated several times and gave always the same sort of difference.

Exposure to light before or after diluting causes a fading of the color. For our tests we used a 500-Watt daylight Mazda bulb. The solutions were kept about a foot from the bulb. The temperature in no case increased more than 0.3° C. For example ten minutes exposure before diluting gave a reading of 16.4 against the control at 15. Exposure for five minutes after diluting gave a reading of 16.4, 16.8 against the control at 15. The exposed solution was definitely gray.

The addition of xylol or toluol in minute amounts for two days at refrigeration temperatures had no appreciable effect upon the final blue color.

Readings on high or low sugars against the glass disc cannot be regarded as accurate any more than against a standard solution of like intensity. The means to solve this difficulty is the usual colorimeter correction curve which must be prepared by the individual workers.

## II NITROGEN STANDARD\*

The ammonium sulphate standard, containing 1 mg N in 100 ml as used, is employed for Urea N and total N in urine, for Urea N and nonprotein N in blood, for proteins in milk, and for any Micro Kjeldahl method where Nesslerization is the final step

The chemicals used in the preparation of the solution for standardizing the glass follow

Ammonium sulphate	- Baker's Analyzed
Nessler's Solution	- (Bock Benedict formula)
Mercuric iodide	- Merck's, Blue Label
Potassium iodide	- Merck's Blue Label
Sodium hydroxide	- Merck's Blue Label

The yellow color obtained is remarkably stable. It darkens noticeably the first half hour after preparation. After this the color intensity remains practically the same for hours. On prolonged standing the ammonium sulphate standard acquires an orange tint which makes matching with unknowns somewhat difficult. The unknowns we have tested show no appreciable increase in color on standing after Nesslerization.

The standard solution darkens slowly so that after eighteen hours its reading against the glass standard will have dropped 0.5 to 1.0 mm, and the orange tint may be very pronounced. The addition of 1 drop of 10 per cent HCl and 2 drops of amyl alcohol either alone or together had no apparent effect upon the orange color.

Since exposure of the nitrogen standard in an open beaker to a 500 watt daylight bulb deepens the color appreciably, we recommend that the solution be kept away from strong light whenever the worker checks the glass standard against his standard solution. The glass is used without plunger or cup.

Since the standard solution does not read 15 against the glass a corrective factor is necessary. If possible each worker should determine his own. Where this is out of the question we suggest 0.95 or 0.96 as the corrective factor. The reading of the unknown is multiplied by this factor and the corrected reading applied to the regular calculation. See Tables III and IV.

TABLE III  
NITROGEN STANDARD

MINUTES AFTER PREPARATION	READING	REMARKS	CORRECTIVE FACTOR
2	16.0 16.1	Glass a little too yellow	0.94 0.93
4	16.0		0.94
9	15.7		0.95
14	15.7		0.95
40	15.6	Faint orange in standard	0.96
55	15.3 1.		0.98 0.97
140	15.4 15.5		0.97
155	15.6	Distinctly orange	0.96
18 hours	14.0	Very orange against yellow glass	1.07

The methods used for nonprotein nitrogen and urea in blood are those described by Victor C. Myers in *Practical Chemical Analysis of Blood* 1924 p. 43-46.

TABLE IV  
UREA ROUTINE

BLOOD NO	TIME AFTER NESSLERIZATION	COMPARISON AGAINST GLASS		COMPARISON AGAINST SOLUTION	
		P	CALCULATED VALUE CF = 0.96	P	CALCULATED VALUE MG/100 cc
Standard	35 minutes	15.5	CF = 0.96		
1	10 minutes	16.1	14.7	15.0	15.0
2	17 minutes	22.6	10.4	21.4	10.5
3	20 minutes	23.0	10.2	22.2	10.1
4	22 minutes	18.8	12.5	17.7	12.7
5	25 minutes	15.2	15.6	14.6	15.4
6	27 minutes	17.8	13.2	17.0	13.2
11	30 minutes	16.7	14.0	16.2	13.8
12	32 minutes	28.6	8.2	27.0	8.3
11	again after 2 hours	16.5	14.2		
1	again after 1 hour	16.0	14.7		

### III URIC ACID\*

For our purpose we employ the method of Brown<sup>4</sup>. Two standards, 0.05 and 0.025 are necessary. The blue color here varies rapidly.

With a method as simple as this, we see no reason for attempting to run a long series at the same time. Two or three prepared at five or ten minute intervals can be matched against the glass standards with as great accuracy as is possible with any standard solution. We strongly recommend that the period of color development be increased to twenty-five minutes instead of twenty unless turbidity intervenes. At the end of the twenty-minute period we have invariably found the standard solutions still increasing rapidly in color. The best time for matching is about thirty minutes after starting color development. From about twenty-six to thirty-three minutes after the start, the standard solutions oscillate about 15 when compared to their proper glass standards. The plunger is best used with the glass, otherwise there will be too much "shade" in the unknown solution.

If color comparison is made at the end of the twenty minutes, a corrective factor is necessary. (Consult Tables V, VI, VII.) There is a progressive deepening of color until the solution becomes too gray to match.

Exposure to light causes a fading of the blue color.

TABLE V  
URIC ACID 0.05 STANDARD

TIME AFTER SETTING UP	READING	REMARKS	CORRECTIVE FACTOR
20 minutes	16.8 17.0	Good match	0.89 0.88
22 minutes	15.8		0.95
24 minutes	15.1		0.99
26 minutes	15.1 15.4		0.99 0.97
28 minutes	15.2		0.99
30 minutes	14.8 15.0		1.01 1.00
32 minutes	15.0		1.00
34 minutes	14.3	Harder to match	1.05

\*The author is indebted to Dr. Ichiro Katayama, Claire Murphy, and Lena Halpern for preparation of the standard solution.

TABLE VI  
URIC ACID 0.05 STANDARD

TIME AFTER SETTING UP	READING	REMARKS	CORRECTIVE FACTOR
20 minutes	17.0	Good match	0.88
20½ minutes	16.7		0.90
21 minutes	16.3		0.92
21½ minutes	15.9		0.94
22 minutes	15.8		0.95
23 minutes	15.7		0.95
24 minutes	15.2		0.99
25 minutes	15.1		0.99
26 minutes	15.0		1.00
28 minutes	14.8 15.0		1.01 1.00
29 minutes	14.8 15.0	Harder to match	1.01 1.00
30 minutes	14.8		1.01
32 minutes	14.8		1.01

TABLE VII  
URIC ACID 0.025 STANDARD

TIME AFTER SETTING UP	READING	REMARKS	CORRECTIVE FACTOR
19 minutes	16.8 17.0	Poor match	0.89 0.88
22 minutes	15.9		0.94
24 minutes	15.8		0.95
26 minutes	15.4	Good match	0.97
28 minutes	15.3		0.98
30 minutes	15.2 15.4		0.99 0.97
31 minutes	15.2		0.99
33 minutes	15.2	Harder to match	0.99
34 minutes	15.1		0.99
36 minutes	14.8		1.01
38 minutes	14.4		1.04
41 minutes	14.0	Very hard to match	1.07
44 minutes	13.9		1.08

# SUMMARY

Glass standards for blood sugar, urine and blood nitrogen and blood uric acid have been described and their limitations noted.

The glass standards described offer a solution of the standard problem, albeit with reservations, and what is more important, they offer to the laboratory investigator the means of studying the color changes and their variations in the methods upon which he depends.

The author is indebted to Mr. Klett and to his assistant Mr. Daniel for their untiring efforts in preparing dozens of trial glasses for these experiments.

# REFERENCES

1. Folin O. The Determination of Sugar in Blood and in Normal Urine. *J. Biol. Chem.* 67: 357, 1926.
2. Folin O. and Wu H. A System of Blood Analysis. Supplement I. A Simplified and Improved Method for Determination of Sugar. *J. Biol. Chem.* 41: 361, 1920.
3. Wu H. Contribution to the Chemistry of Phosphomolybdic Acids, Phosphotungstic Acids and Allied Substances. *J. Biol. Chem.* 43: 206, 1920.
4. Brown H. The Determination of Uric Acid in Blood. *J. Biol. Chem.* 68: 123, 1926.

# STUDIES ON ANTIGEN FOR THE KAHN TEST

## II A SENSITIZING REAGENT

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ANTIGENS used in serum tests for syphilis may be said to have two outstanding limitations. In the first place, they lack uniformity in sensitiveness, even when a standard method of preparation is used, this is due to the fact that the heart muscle from which the antigen is prepared varies markedly in its content of antigenic lipids. In the second place, antigens appear in general to be insufficiently sensitive, no antigen thus far prepared being capable of detecting all cases of syphilis. The development of antigens more adequately adjusted to diagnostic ends has been somewhat delayed by a meager knowledge of the factors governing antigen sensitiveness. During the past several years the studies on antigen for the Kahn test, carried out in this laboratory, have been directed toward overcoming both of these limitations. A preliminary report dealing with the uniformity of standard antigen has recently appeared.<sup>1</sup> The present study deals with two series of experiments on antigen sensitiveness. In the first series, attempts were made to determine the relative amount of antigenic material present in the discarded by-products of antigen preparation. The results obtained in these first studies were then applied in a second series of experiments in the course of which a sensitizing reagent was developed capable of increasing antigen sensitiveness without apparently affecting specificity.

It will be recalled<sup>2</sup> that the preparation of antigen for the Kahn test involves two basic extractions of powdered heart muscle, a series of extractions with ether followed by a single extraction with alcohol, and that it is this alcohol extract which, after cholesterolization and standardization, is employed as the antigen. It was early observed that the extent of the ether extractions of heart muscle markedly affects the sensitiveness of the final product. Excessive extraction with ether tends to reduce antigen sensitiveness, while deficient extraction with ether, within certain limits, increases antigen sensitiveness. Thus in the preparation of standard antigen, 25 gm of heart muscle are extracted successively with 100, 75, 75, and 75 cc of ether, while in the case of the "special" more sensitive antigen that was prepared for use in the presumptive procedure, the successively employed ether amounts for the same quantity of heart muscle were 50, 50, 40, and 40 cc. Since much of the lipid material that would be removed by a preliminary ether treatment will go into solution in the alcohol if the ether treatment is omitted or reduced, the antigen prepared according to the second, "special" method contains more ether extractives than the first, and is therefore

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more sensitive. It was further reported from earlier experiments than when 25 gm of heart muscle are extracted with larger volumes of ether than those employed in the preparation of standard antigen, the resulting reduction in the sensitiveness of the antigen can be overcome by adding to the antigen ether extractives in concentrated form. Such extractives can also be utilized in collecting antigens which are similarly less sensitive than standard antigen because of inherent poverty in antigenic lipids of the heart muscle from which they are prepared.

The fact that the ether extractives of heart muscle contain antigenic lipids prompted a study of these extractives with a view to their utilization in the Kahn test. At first it seemed desirable to prepare an acetone insoluble extract of these ether extractives and to test its degree of sensitiveness and specificity with syphilitic and nonsyphilitic sera. The antigenic value of a secondary alcoholic extract of heart muscle seemed also worth investigating. Finally, it seemed desirable to prepare an alcoholic extract of ether extractives. The studies carried out with this last extract ultimately led to the preparation of an antigen sensitizing reagent which under proper conditions is capable of increasing antigen sensitiveness.

#### I. ANTIGENIC VALUE OF THREE SPECIAL EXTRACTS

*A. An Acetone Insoluble Fraction of the Ether Extractives of Heart Muscle*—The ether extractives obtained in the preparation of antigen from 50 gm powdered beef heart were evaporated nearly to dryness. The small amount of water which collected at the bottom of the evaporating dish was removed by means of a capillary pipette and the lipid residue was then dissolved in 15 c.c. of ether and the solution filtered. When 10 c.c. of the filtrate were measured into a 250 c.c. cylinder and 200 c.c. acetone added, a fine precipitate appeared instantly. After vigorous shaking the particles became larger and soon settled to the bottom of the cylinder leaving the acetone solution practically clear. The supernatant acetone was decanted, and the lipid residue taken up in 50 c.c. of 95 per cent alcohol. Extraction was permitted to take place for thirty minutes at room temperature with frequent shaking and the alcoholic mixture was then filtered. To the filtrate were added 6 mg cholesterol per cubic centimeter, the cholesterol being dissolved by warming the mixture in a water bath. After filtration, the extract was titrated, employing the same method as is used for the titration of standard antigen. It was then tested with syphilitic and nonsyphilitic sera.

Table I illustrates the comparative sensitiveness of the acetone insoluble fraction of the ether extractives and of standard antigen. It is evident from this table that the fraction not only has antigenic properties but is actually comparable in sensitiveness and specificity with standard antigen. It was noticed, however, that the negative reactions appeared more cloudy than in the case of standard antigen. An attempt was made to overcome this short coming by a readjustment of the amount of salt solution used in preparing the antigen suspension. When, however, the titer was increased to 1 + 21 the negative reactions still appeared somewhat cloudy, and furthermore it



TABLE I  
COMPARATIVE SENSITIVENESS OF ACETONE  
INSOLUBLE FRACTION OF ETHER EXTRACTIVES, AND OF STANDARD ANTIGEN

SERUM NO	ACETONE INSOLUBLE FRACTION TITER 1 + 1 9			STANDARD ANTIGEN TITER 1 + 1 1		
	1	2	3	1	2	3
1	—	—	—	—	—	—
2	—	—	—	—	±	++
3	—	—	±	—	—	—
4	—	±	±	—	—	—
5	++	++	+++	+++	++++	++++
6	++	++++	++++	++++	++++	++++
7 - 10	++++	++++	++++	++++	++++	++++

was observed that the antigen gave atypical reactions with four-plus sera, or reactions in which the third tube of the regular test showed very weak precipitates. The results are illustrated in Table II.

When the acetone insoluble fraction was diluted with cholesterolized alcohol (10 to 25 per cent dilution), there was observed a slight increase in sensitiveness. When the fractions were added, either undiluted or after dilution with cholesterolized alcohol, to standard antigen in a 50/50 proportion, the sensitiveness of the final mixtures was not markedly different from that of standard antigen, except that the antigen containing the acetone insoluble fractions gave atypical reactions.

TABLE II  
ATYPICAL REACTIONS GIVEN BY ACETONE INSOLUBLE FRACTION

SERUM NO	ACETONE INSOLUBLE FRACTION TITER 1 + 2 1			STANDARD ANTIGEN TITER 1 + 1 1		
	1	2	3	1	2	3
1	—	—	—	—	—	—
2	++++	++++	±	++++	++++	++++
3	++++	++++	+	++++	++++	++++
4	++++	++++	+++	++++	++++	++++
5	++++	++++	++	++++	++++	++++
6	—	—	—	—	—	—

The results obtained with the acetone insoluble fractions of the ether extractions did not seem to warrant further detailed studies of this fraction, because, although it possesses a degree of sensitiveness approximating that of standard antigen, it is somewhat troublesome to prepare, it does not appear to give a sharp titer, and it has a tendency to give atypical reactions.

*B A Secondary Alcoholic Extract of Heart Muscle*—Our next problem was to determine whether a secondary alcoholic extract of powdered heart muscle that had already been treated to the first alcoholic extraction in the standard preparation of antigen, would show antigenic properties. This had been attempted before with negative results,<sup>2</sup> but it seemed worth while to repeat the experiments. Powdered beef heart was used from which the original (primary) alcoholic extract had been filtered without the aid of suction through qualitative filter paper in a glass funnel. This method of filtration permitted a considerable amount of the alcoholic filtrate to adhere

to the residue. For the secondary extract, 50 gm of this residue were extracted with 100 cc of 95 per cent alcohol for one hour, in a water bath at 60° C, and finally for twenty four hours at room temperature. When this secondary extract was allowed to stand after filtration, a precipitate separated slowly from the liquid and was removed by repeated filtrations. After five days standing, the extract showed only a trace of a precipitate and was then cholesterolized, 6 mg of cholesterol being dissolved in each cubic centimeter of extract.

The secondary alcoholic extract was titrated in the usual manner and tested for sensitiveness with syphilitic sera of different potency, standard antigen being used as a control. The results with several sera are given in Table III. It is evident from this table that the secondary alcoholic extract was considerably less sensitive than standard antigen.

TABLE III

COMPARATIVE SENSITIVENESS OF SECONDARY ALCOHOLIC EXTRACT OF HEART MUSCLE AND OF STANDARD ANTIGEN

SERUM NO	SECONDARY ALCOHOLIC EXTRACT TITER 1 + 12			STANDARD ANTIGEN TITER 1 + 11		
	1	2	3	1	2	3
1	++++	++++	++++	++++	++++	++++
2	-	±	±	+++	++++	++++
3	-	±	-	+	++	++
4	±	+	+	-	+	++
5 - 6	-	-	-	-	-	-

The question arose as to whether the antigenic powers of the secondary alcoholic extract were not in part due to the occlusion of some of the original extract antigen in the beef heart residue. At least 30 per cent of the alcoholic filtrate remains adhering to the particles when the filtration process is carried out in an ordinary glass funnel. In order to throw light on this question, standard antigen was prepared from 100 gm of beef heart, and was filtered as completely as possible by means of suction through a Buchner funnel. To the powder residue were then added 250 cc of alcohol. Extraction was carried out as previously described, namely, for one hour at 60° C followed by twenty four hours at room temperature. After filtration and cholesterolization this secondary extract was practically free from antigenic properties. As will be shown later when "sensitizing reagent" was added to this secondary alcoholic extract, the resulting product showed some antigenic properties with syphilitic serum. The precipitates were however, extremely fine and difficult to detect.

To summarize our findings with secondary alcoholic extracts of heart muscle, it appears that such extracts have very little antigenic material, especially when the heart muscle has previously been freed from the original antigen by filtration with suction.

*C An Alcoholic Extract of Ether Extractives of Heart Muscle*—Our studies were next directed to the preparation of an alcoholic extract of the concentrated ether extractives of heart muscle. About 500 cc ether extract

collected in the course of antigen preparation from 50 gm of powdered beef heart, were evaporated nearly to dryness with the aid of an electric fan. When practically all of the ether had evaporated, a few globules of water, which separated from the lipid mass and settled to the bottom of the dish, were removed with a capillary pipette. The lipid residue was taken up in 50 cc of absolute alcohol, and the mixture was heated for ten minutes in a water bath at 56° C with frequent shaking. The mixture was then placed in the ice box (7° C) for one hour, and was filtered while cold. The clear yellow solution became cloudy after standing about one-half hour at room temperature. It was refiltered and cholesterolized (6 mg of cholesterol per cc).

It was observed that this extract was of little practical value when used directly as an antigen. Indeed, it was impossible to establish its "titer." Even when as much as 3 to 5 cc of salt solution were added to 1 cc of the extract, there resulted a lipid suspension the particles of which were not entirely dispersible in additional salt solution or in negative serum. When, however, the extract was added to standard antigen in a proportion of about 2 or 3 parts of extract to 100 parts of antigen, it markedly increased the sensitiveness of the latter. Table IV gives results of comparative tests with standard antigen alone and standard antigen plus 3 per cent of the special extract.

TABLE IV

EFFECT ON SENSITIVENESS OF STANDARD ANTIGEN OF ADDING ETHER SOLUBLE, ALCOHOL SOLUBLE EXTRACT

SERUM NO	STANDARD ANTIGEN PLUS 3% ETHER SOLUBLE, ALCOHOL SOLUBLE EXTRACT			STANDARD ANTIGEN		
	TITER 1 + 20			TITER 1 + 11		
	1	2	3	1	2	3
1 - 2	-	-	-	-	-	-
3	-	++	++++	-	-	±
4	+	++++	++++	-	-	++
5	++	++++	++++	-	+	++
6	++--	++++	++++	-	++	++++
7	+++	++++	++++	-	++	+++
8	++--	++++	++++	±	++++	++++
9 - 10	++++	++++	++++	+	++++	++++

This observation suggested that the preparation of an alcoholic solution of the ether extractives in a stable form would yield a valuable reagent for the Kahn test. A reagent of this type ("sensitizing reagent") could be used for correcting newly prepared antigens that are less sensitive than standard, it could also be used in the preparation of antigens more sensitive than standard.

## II EXPERIMENTS ON PREPARATION OF SENSITIZING REAGENT

In attempting to standardize the method of preparing the sensitizing reagent many procedures were tried. Ten minute alcohol extractions of the lipid residue from the ether extract were carried out in some cases at 56° C, and in other cases at 60° C. These extractions were followed by further

extraction in the ice box for from one to three hours. Although the resulting reagents showed good promise with respect to their antigen sensitizing properties, this method of preparation was ultimately abandoned because the extracts tended to yield precipitates on standing. These precipitates usually appeared in the form of a gummy sediment adhering to the walls of the container. On filtration, a new sediment would appear after several days' standing. It was not possible to determine whether the sediment had antigenic properties, since so small a quantity was deposited. The extract showed, however, but little variation in its sensitizing properties after repeated filtration from the sediment. Nevertheless it was believed undesirable to accept as standard a method of preparation yielding a sensitizing reagent from which a sediment was constantly being deposited. Attempts were therefore made so to modify the method as to prepare an effective sensitizing reagent that would remain free from a precipitate.

Various modifications of the extraction procedure were tested. The lipid residue from the ether extract was extracted with alcohol for ten minutes at room temperature (instead of at 56° C. or 60° C.) followed by several hours in the ice box (7° C.). In other cases tried the extraction for ten minutes at room temperature was followed by continued extraction in the ice box for three days. In still other cases the residue was extracted with alcohol at room temperature for three days. All these methods yielded sensitizing reagents from which sediments were deposited when the reagents were allowed to stand for several days. *It was finally observed that the formation of a sediment was either markedly reduced or entirely eliminated if after the extraction of the lipid residue at about 7° C. the mixture was filtered and the filtrate placed in the incubator at 37° C. overnight.* This observation led to the adoption of the following extraction method. The residue is extracted at room temperature for ten minutes and then in the ice box at about 7° C. for three or four hours. The mixture is filtered while cold and the filtrate placed in the incubator at 37° C. for twenty-four hours.

Many varieties of heart muscle were found to be satisfactory for use in the preparation of sensitizing reagent. Several lots of "Difco" powdered heart muscle were employed with good results. Home ground sheep heart, pig heart, and beef heart were also found to produce good sensitizing reagents. The concentrated ether extract of pig heart contained a colorless fluid which collected in a layer above the usual brown residue and which could apparently be removed without interfering with the sensitizing properties of the final alcoholic extract.

The evaporation of the ether extractives was in most cases carried out with the aid of an electric fan. In several instances however an electrically heated water bath at 100° C. was employed for this purpose while in isolated cases fan and electric water bath were combined and the evaporation of the ether took place very rapidly. All these methods of evaporation appeared to give good results. In two instances the highly viscous lipid residue obtained after evaporating the ether was left overnight thus permitting ox

dation and partial solidification of the lipids. This treatment did not, however, interfere with the production of a good sensitizing reagent.

The treatment of the lipid residue after evaporation of the ether was also subjected to special study. In some cases, the globules of water which collected at the bottom of the evaporating dish were removed with a capillary pipette. In other cases, the water was permitted to evaporate on a water-bath at 100° C. In some instances the mixture of lipid residue and water was poured into a centrifuge tube, and after about five minutes' centrifugalization, the water which had collected at the bottom of the tube was readily removed with a capillary pipette. All these methods were found to give comparable results.

With regard to the alcohol used for the extraction process, absolute alcohol was found to be superior to 95 per cent alcohol, the former producing more sensitive sensitizing reagents. A proportion of 10 c.c. of absolute alcohol per gram of residue (or 8 c.c. absolute alcohol per cubic centimeter of residue) was found to be satisfactory. Extraction proportions of 20 or 15 c.c. of alcohol per gram of residue produced reagents which were less sensitive.

### III. METHOD OF PREPARING SENSITIZING REAGENTS

On the basis of these experiments the following method was adopted for the preparation of sensitizing reagent:

- 1 The ether filtrate obtained as a by-product in the preparation of antigen from 25 gm. of heart muscle (about 250 c.c. of filtrate, allowing for some loss by evaporation) is refiltered to remove traces of powdered muscle, and the solvent is then evaporated with the aid of an electric fan.

- 2 When the volume has been reduced to about 10 c.c., the concentrated ether extract is transferred to a small, weighed evaporating dish (capacity about 20 c.c.), the transference being made complete by washing out the residue into the small dish with a little ether.

- 3 Evaporation is continued with the aid of the fan until there remains but a slight trace of ether odor.

- 4 At this stage there may separate from the dark brown lipid mass a few cubic centimeters of water. This water, which will be at the bottom of the evaporating dish, is removed by means of a capillary pipette. The lipid residue is brownish, semitransparent, and viscous\*.

- 5 As soon as the residue is completely free from ether odor, the evaporating dish is reweighed, and the weight of the residue determined.

- 6 The residue is transferred to an Erlenmeyer flask (about 100 c.c. capacity). This is best accomplished with the aid of a small spatula.

- 7 A volume of absolute alcohol equivalent to 10 c.c. per gram of residue is added to the flask, a small amount of this alcohol being employed for rinsing the evaporating dish.

\*An alternate method is to remove the water at this stage by evaporation for ten minutes on a water-bath at 100°C (stirring with a glass rod). After evaporation steps 5 and 6 etc. follow as outlined, but in step 7 the volume of absolute alcohol used should then be 13 c.c. instead of 10 c.c. per gm. of heated residue.

8 Extraction is allowed to take place for ten minutes at room temperature with frequent shaking of the flask. Only a fraction of the residue dissolves in alcohol, lipid masses being distributed throughout the mixture.

9 The mixture is placed in the ice box ( $4^{\circ}$  to  $9^{\circ}$  C) for three hours, during which period the bulk of undissolved matter is increased by the formation of a white precipitate.

10 The mixture is filtered while cold and the flask containing the clear dark yellow filtrate is placed in the incubator at  $37^{\circ}$  C for twenty-four hours.

11 The clear filtrate is permitted to stand for three days at room temperature. If a precipitate forms during this test period the solution is refiltered and again incubated for twenty-four hours at  $37^{\circ}$  C.

12 The filtrate is cholesterolized with 6 mg cholesterol per cc according to the usual technique.

13 The cholesterol extract, known as 'sensitizing reagent' is filtered and is then ready for use.

#### IV. PROPERTIES OF SENSITIZING REAGENT

Sensitizing reagent has the property of increasing the sensitiveness of antigens that are less sensitive than standard, it can also increase the sensitiveness of standard antigen. When the amount of reagent added to antigen is less than 1 per cent, the increase in sensitiveness is usually small. As the proportion of reagent to standard antigen is increased to 2, 3, 4, and 5 per cent, the products show a graded increase in sensitiveness. Increasing the amount of reagent beyond 5 per cent usually results in a drop in the sensitiveness of the final product. This is illustrated by the following experiment.

To five 10 cc quantities of standard antigen were added 1.5, 3, 6, 9, and 12 per cent sensitizing reagent respectively. The modified antigens were titrated in the usual manner and were tested comparatively employing the 3 tube Kahn test. It was found that the addition of 1.5, 3, and 6 per cent sensitizing reagent to standard antigen resulted in a progressive increase in sensitiveness while the addition of 9 and 12 per cent sensitizing reagent resulted in a progressive decrease in antigen sensitiveness. The results of the experiment are shown in Table V.

TABLE V

EFFECT ON THE SENSITIVENESS OF STANDARD ANTIGEN OF ADDING INCREASING AMOUNTS OF SENSITIZING REAGENT\*

NO SERUM	PARTS OF SENSITIZING REAGENT ADDED TO 100 PARTS OF STANDARD ANTIGEN					
	0 TITER 1 + 11	1.5 TITER 1 + 10	3 TITER 1 + 21	6 TITER 1 + 23	9 TITER 1 + 25	12 TITER 1 + 27
1	++++	++++	++++	++++	++++	+++
2	+++	++++	++++	++++	++++	++++
3	++	+++	++++	++++	+++	++
4	+	++	+++	++++	++	+
5	±	+	++	+++	+	-
6	±	+	+	+++	+	-
7	±	+	+	+++	+	-
8	-	+	+	++	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-

Average of 3 Tube Kahn Test

It is interesting to note that the increase in sensitiveness effected by adding sensitizing reagent to an antigen is often enhanced by diluting the product with cholesterolized alcohol. Table VI illustrates this point, a striking increase in sensitiveness being obtained after adding 1 per cent sensitizing reagent and diluting 10 per cent with cholesterolized alcohol, but only a moderate increase in sensitiveness when 1 per cent sensitizing reagent was added alone.

TABLE VI

COMPARATIVE EFFECT ON ANTIGEN SENSITIVENESS OF ADDING SENSITIZING REAGENT ALONE AND OF ADDING SENSITIZING REAGENT PLUS CHOLESTEROLIZED ALCOHOL

SERUM NO	UNMODIFIED ANTIGEN (UNDERSENSITIVE)			ANTIGEN MODIFIED BY ADDITION OF 1 PER CENT SENSITIZING REAGENT			ANTIGEN MODIFIED BY ADDITION OF 1 PER CENT SENSITIZING REAGENT PLUS 10 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL		
	TITER 1 + 1			TITER 1 + 11			TITER 1 + 11		
	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	±	++
3	-	-	±	-	-	±	-	-	++
4	-	-	±	-	±	+	±	++	+++
5	-	±	+	-	±	+	±	+++	+++
6	-	±	+	-	±	++	±	+++	+++
7	-	±	+	-	++	++	-	++	+++
8	-	±	+++	±	+++	+++	+++	+++	+++
9	++	+++	++++	-	+++	++++	+++	+++	+++
10	+++	++++	++++	+++	++++	++++	++++	++++	++++

It was stated above that the secondary alcoholic extract has but slight antigenic properties. If, however, sensitizing reagent is added to the secondary alcoholic extract in suitable proportions, the final mixture can be rendered even more sensitive than standard antigen. This is illustrated in Table VII.

TABLE VII

EFFECT OF ADDING SENSITIZING REAGENT TO THE SECONDARY ALCOHOLIC EXTRACT OF HEART MUSCLE

SERUM NO	SECONDARY ALCOHOLIC EXTRACT PLUS 2 PER CENT SENSITIZING REAGENT			STANDARD ANTIGEN		
	TITER 1 + 20			TITER 1 + 11		
	1	2	3	1	2	3
1	-	-	-	-	-	-
2	-	±	+	-	±	+
3	++	+++	+++	±	+	+++
4	++	++++	++++	+	++++	++++
5	+++	++++	++++	++	++++	++++
6	++++	++++	++++	++	++++	++++
7	+++	++++	++++	+++	++++	++++
8-9	++++	++++	++++	++++	++++	++++

It should be mentioned, however, that the precipitates obtained in the positive reactions were unusually fine, suggesting an insufficiency of lipids in the mixture.

## SUMMARY

Studies were undertaken to determine whether the by-products obtained in the preparation of antigen for the Kahn test could be utilized for preparing

a stable antigenic solution. An acetone insoluble product of the concentrated ether extractives of heart muscle showed antigenic properties, but when used alone was less satisfactory than standard antigen, and when added to standard antigen did not improve the antigenic qualities of the latter. A secondary alcoholic extract of heart muscle was found to be relatively poor in antigenic properties. An alcoholic extract of the concentrated ether extractives of heart muscle was found to be unfit for direct use as an antigen in the Kahn test, but on the other hand it was observed that this extract has the property of increasing the sensitiveness of other antigens when mixed with them in suitable proportions. This extract, known as 'sensitizing reagent,' renders comparatively simple the correction of antigens that are less sensitive than standard. It also renders possible the preparation of antigens more sensitive than the standard.

## REFERENCES

- 1 Kahn, R. I., Nagel, N. and Hendrick, J. J. Studies on Antigen for the Kahn Test, *J. Infect. Dis.* 41: 111-123, 1921.
- 2 Kahn, R. I. Serum Diagnosis of Syphilis by Precipitation, 1924, pp. 68, 121.
- 3 Ibid. pp. 117, 210.
- 4 Kahn, R. I. The Kahn Test. A Practical Guide, 1928, p. 141.

## A FILLING DEVICE FOR MICRO PIPETTES\*

BY HARRY D. TRIPP, M.B. CHICAGO, ILL.

EVERY person who has used pipettes of small caliber is familiar with the tediousness encountered in their usage. The tiny column of fluid is difficult to control by the customary method of applying oral suction by way of a rubber tubing and mouth piece. This method is, of course, also very unsanitary. Several attempts are sometimes necessary in order to have the column stop on the proper calibration. This is especially true with blood counting pipettes.

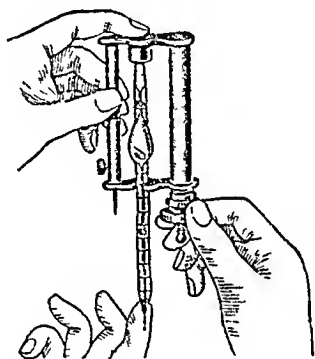
Other objections to the oral method of filling these pipettes are: (1) By gravitation a certain amount of saliva will find its way into the rubber tubing, and then into the pipette, thus contaminating the pipette and the fluid being drawn into it. (2) Pinching off of the rubber tubing to stop the column may expel a portion of the contents. (3) The danger of infection should some of the bloody mixture, serum or bacterial suspension, be accidentally drawn into the mouth. It is also possible for the rubber tubing to become contaminated after numerous blood counts have been made, and as a rule one does not think to sterilize these tubes before placing them in the mouth. (4) The usual method of blowing one's breath through these pipettes in order to force cleaning fluids through them is not a good one because the breath is the last substance blown through. It condenses on the inner walls of the pipette so that actually the pipette is not clean when the usual cleaning procedure is complete.

To overcome some of the difficulties I have arranged a device adaptable



to small pipettes, such as the blood counting pipettes, the Folin-Wu and Byrd pipettes used in blood sugar analyses, and other pipettes as used in the Kahn syphilis test, and those used in making bacterial counts. It is a small, compact device carrying in the same structure an adjustable puncture needle. Pipettes are readily attached and replaced, and facilities for cleaning them are inherent. To operate, one simply holds the device in the hand and manipulates a milled head which perfectly controls the column in the pipette so that specific quantities can be drawn into the pipette or expelled from it. The device is so arranged that after filling the pipette it is held so firmly that agitation of the contents is accomplished by shaking the whole device without removing the pipette.

A few suggestions in the technic of taking blood samples may be helpful as it may appear that this device cannot be used when taking blood from the ear. However, it is not as difficult as it may seem. Usually some inconvenience is experienced because the puncture is made too deep and the blood runs down



the patient's neck or the puncture is not made deep enough and then an attempt is made to express blood by pinching the ear. This is not proper as tissue serum is expressed with the small quantity of blood and if a count is made it will be inaccurate. It is much more convenient to make a sufficiently deep puncture and then collect a few drops of the blood on a clean glass slide which has a small circle drawn on it with a wax pencil. The blood must be placed within the circle to keep it from spreading. To fill a pipette from this then is very easy and much more accurate. The blood will not clot before one fills the pipette, because with the device only a second is sufficient to fill the pipette to the desired mark with blood. A pledget of cotton can be placed on the bleeding ear.

When taking blood from the finger tip I have found it very helpful to loop a small rubber band around the finger tip two or three times after stripping the finger. By doing this only a slight prick will bring blood without further pinching of the finger.

With this device and the few points in technic considerably less experience is required to become efficient in making blood counts and other blood examinations, it simplifies the process of diluting the blood and would appear to be more accurate than the customary method.

# AN IMPROVED ANIMAL CAGE FOR SCIENTIFIC INVESTIGATIONS

By A. RICHARD BLISS,\* J. PHIM D. M.D., MEMPHIS, TENN.  
AND GEORGE S. BLISS † B.S., NEW YORK CITY

THE cages, described herein and used in the laboratories of the University of Tennessee Colleges of Medicine and Dentistry and School of Pharmacy at Memphis during the past college session, have proved very satisfactory. Long experiences with stock cages and with cages constructed in the Shop of the University of Tennessee, all of which showed some marked disadvantages from the standpoints of animal comfort, care, accurate work and costs, resulted in the invention of the cages under discussion.

These cages, which may be built to any desired dimensions, provide isolating compartments for different sizes of dogs, cats, rabbits, monkeys, or other small animals. The cages are constructed so as to furnish maximum ventilation and visibility, while affording complete comfort to caged animals. The high degree of comfort provided is conclusively shown by the fact that large dogs which have been confined by us in cages of this type 3 feet x 3 feet x 3 feet, have given none of the usual noisy evidences of discomfort and fretfulness.

The cages are constructed of sheet metal (filing cabinet steel) which is covered with baked enamel of desired color and they present a most pleasing appearance. Fig. 1 presents a general view of one of the cages. The back and sides are of steel plate with heavy wire mesh toppings. The mesh toppings may be made of any desired height. The top of the cage is of steel plate or of wire mesh as desired. The front is a duplex door, the lower unit being a sheet metal door, and the upper a wire mesh door, each provided with a fastening bolt. The sheet metal door carries a large label holder and has an angular upper edge offset laterally inward against which the wire mesh door closes at the bottom. (See Fig. 2.) This arrangement permits close unobstructed observation of the confined animal and the placing of food, water and other materials in the cage with practically no opportunity for the animal to escape.

A water or food tray is carried on the inside of the lower door. (See Fig. 2.) If desired the tray may be fastened to one of the sides. The tray is attached to threaded rods the upper ends of which are squared. By simply turning the squared tops of the rods with the fingers the tray is caused to travel up or down to a height which is within comfortable reach of the particular animal under observation and without the liability of the tray contents being spilled or contaminated by the urine or feces of the animal.

The floor supporting the animal consists of a readily removable and re-

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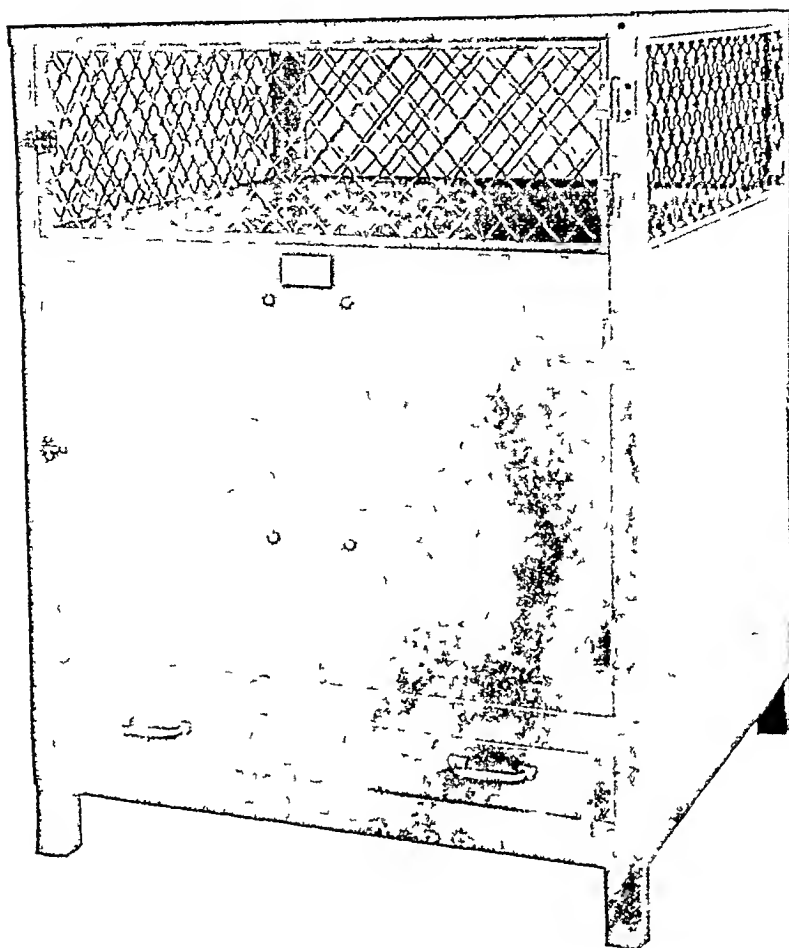


Fig 1—Bliss animal cage

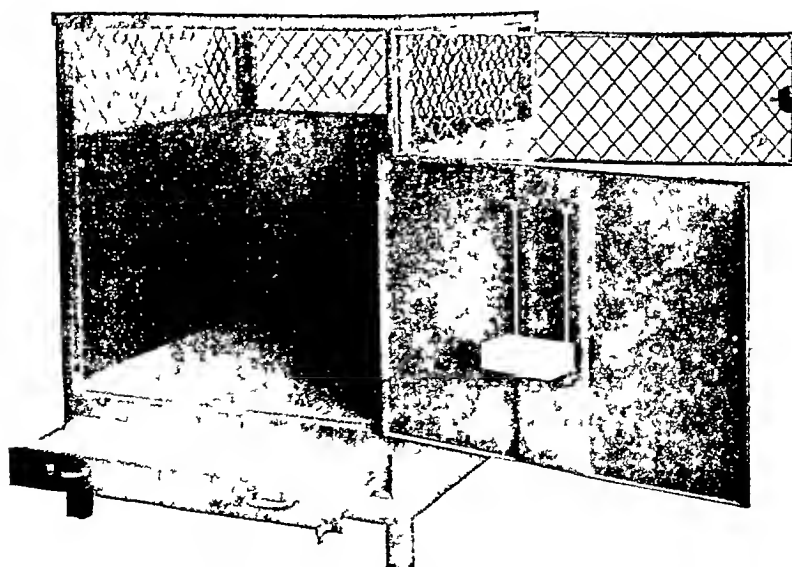


Fig 2—This view shows the duplex door water or food tray false mesh bottom and napper drawer

placeable false bottom of suitable wire mesh, which permits the free passage of the urinal discharges of the animal. The removable floor rests on a ledge which is provided with a flange directed laterally inward and downward to deflect liquid passing through the mesh floor away from the lower sides of the cage body. (See Fig 3)

Under the floor or false bottom is a removable sliding hopper drawer with its inner bottom sloping in all directions toward one outer corner which is provided with a small drain or draw off cock. Accordingly all liquid dis-

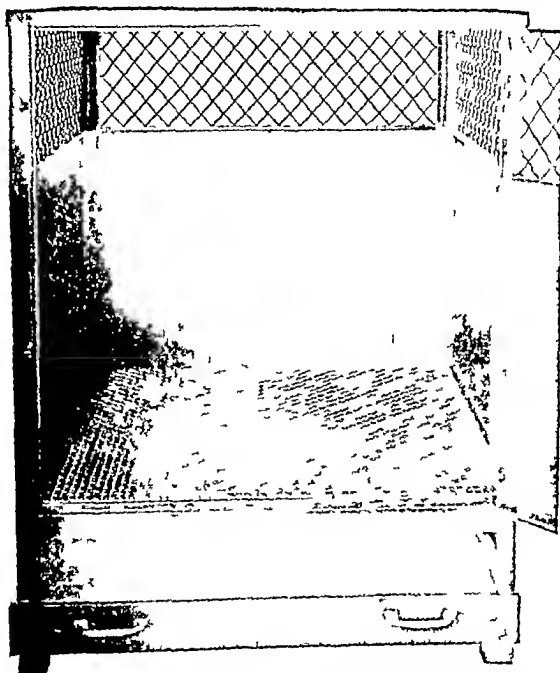


Fig 3—View showing false mesh bottom and hopper drawer carrying drain cock

charge from the animal will pass into the hopper drawer where it is accurately and completely collected, and from which it may be completely drawn off at will. The construction material insures against corrosion and contamination of the collected fluids. (See Figs 2 and 3)

The legs of the cages are removable so that they may be stacked in tiers. (See Fig 4) Castor bases may replace the legs.

The construction and the materials of the cages make them practically indestructible, and permit of easy thorough cleansing and sterilization by dis-

infecting and cleansing agents, boiling water and steam. The weight of the cages permits of easy moving. The cost is surprisingly low.

By an easy modification of the foregoing construction a very inexpensive cage is provided suitable for simple storage and isolation. Full mesh panels

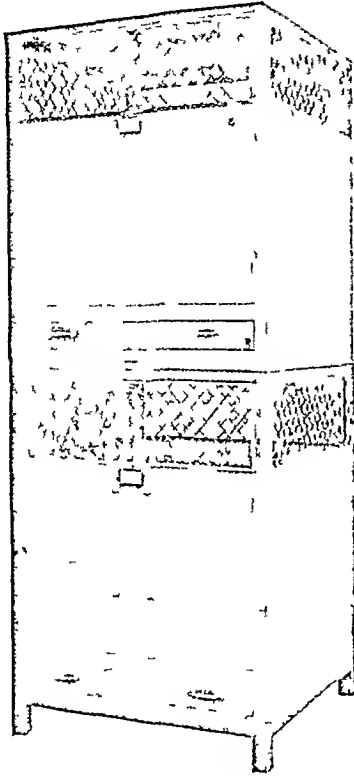


Fig 4 —Cages stacked in tier

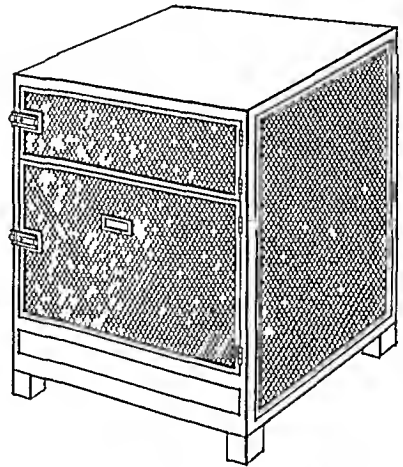


Fig 5 —Simple storage cage

may be used for the sides and door of the cage. The back and the top may be made of solid steel or mesh as desired, and the bottom of solid steel in place of mesh. No hopper drawer is then needed. (See Fig 5.)

## A SIMPLE AND EFFICIENT METHOD FOR PERMANENTLY NUMBERING RABBITS\*

By K. L. McALPINE M.A. (TORONTO) GLENOLDEN, PA

**I**N THE routine assay of insulin a large number of rabbits are required. The assay is greatly facilitated by injecting two groups of nine rabbits, one with the standard insulin and the other with the unknown. One week later the groups are reversed, so that the one which received the standard now receives the unknown, and vice versa. In this manner variables in the susceptibility to insulin of individual animals tend to balance each other.

The above procedure makes it desirable and almost necessary to number permanently each rabbit because after the necessary test period the same



Fig 1

animals may be used repeatedly as long as they weigh between 1800 and 2200 gm. Various forms of tags, clips, and rings have been used with more or less success, but often these produced injury to the ear, thereby interfering with the bleeding. Branding with a toothed punch caused infection at times. Numbers painted on with various dyes and pigments, such as methylene blue, fuchsine, safranin, picric acid, indigo carmine, indelible ink, India ink, etc., although brilliant when applied, soon faded and became obliterated.

To overcome these difficulties the following method has been devised and found very satisfactory. The hammer of a discarded electric bell is bent at right angles to the armature and a sewing needle is soldered to the bent portion. A toy transformer attached to the light circuit may be used as a suitable source of current, although any other 6 volt supply is equally efficient. The

numbers are written on the inner surface of the ear with India ink, and, while the ink is still wet, the vibrating needle is run along the numbers, thus tatooing the pigment into the skin. This process is very rapid, a rabbit may be tatooed with a 3 digit number in about two minutes. The animal experiences little discomfort and no blood is drawn. The numbers put on by this method are permanent. No infections have been observed.

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### A USE FOR ETHER CANS\*

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By MARY B. COOPER, A. B., SYRACUSE, N. Y.

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THE use of quarter-pound anesthetic ether cans to serve as holders for culture tubes dates from the Great War or earlier. The soldered-on cover of the *empty* can is removed by the aid of pliers and of heat from a Bunsen burner.



We have lately adapted the cups thus easily obtained to serve as containers for the small 50 by 13 mm. Petri dishes used for routine blood plates. With tinsmiths' shears a strip about an inch wide is cut out from the rim of the can to the base, care being taken to leave no jagged corners. The plates slip easily in and out of these containers, and are held conveniently during sterilization as well as later when they are filled with media and are in the refrigerator or incubator.

We have found the ether can, which the operating room discards, a useful adjunct to our laboratory.

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\*From the Laboratory of the General Hospital of Syracuse.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D. ABSTRACT EDITOR

## ALCOHOLISM Quantitative Study of Acute Intoxication Bogen E Am J M Sc 76 No 2, 1928

The following methods are proposed by Bogen for the laboratory measurement of alcoholism

### Alcoholic Content of Breath

Reagent (Anstie) A solution of two thirds of 1 per cent of potassium bichromate in concentrated sulphuric acid is diluted with an equal quantity of distilled water and a current of air containing the alcohol in gaseous form bubbled through it. The color change, from reddish yellow to greenish blue is then measured by comparison with a series of standards previously made by the addition of known amounts of alcohol to 5 cc portions of the reagent mixture in sealed tubes

Alcohol in urine, blood spinal fluid tissues etc

Place 1 cc of the specimen in a test tube. A purified air current is bubbled through 5 cc of the reagent for ten minutes while the tubes are immersed in a boiling water bath

When acetone is present in the unknown solution it must be removed by the addition of 1 cc of Scott Wilson's alkaline mercuric cyanide reagent before aeration

The amount of alcohol present can be determined by color comparison with the standards or, more precisely, by titration as follows

To the heated and aerated reagent solution add 3 drops of 1 per cent potassium ferrocyanide and titrate with a standard solution of N/30 ferrous ammonium sulphate solution until a deep blue color appears

Each 2 cc of the standard solution less than 10 cc required for this titration represents 1 mg of alcohol when 1 cc of the unknown is mixed with 5 cc of the reagent

Interpretation border line 1 to 2 mg

Intoxicated 3 mg and over

## ALCOHOLISM Chemical Test For Naville F Rev méd de la Suisse Rom GENEVE 48 849, 1928

If a man or animal ingests absolute alcohol to the extent of 2 or 5 gm per kilo of weight, two hours later there are found 2 to 5 gm of alcohol per liter of blood. Consequently the determination of the alcoholic content of the blood from one to three hours after ingestion will indicate the amount of absolute alcohol which the individual has absorbed per kilo. By multiplying this figure by the weight of the man or animal there will be obtained an amount of absolute alcohol less or equal never more to the amount ingested. If the analysis is made more than three or four hours after ingestion, the figures obtained by the calculation will have to be corrected by taking into account the curves obtained by experimenters

The method of Nicloux is very simple and may be carried out in any laboratory

Ten cc of grams of the substance to be analyzed (blood urine organs) are placed in a distilling flask and about 65 cc of a saturated solution of picric acid are added. The mixture is boiled and the products of distillation are received in a cooled tube until 20 cc have been obtained. Thus 2 cc of the distillate contains the alcohol present in 1 cc of blood or other product analyzed. When distilled in a vacuum all the alcohol is obtained and if an ordinary still and condenser are used about 90 per cent of the alcohol will be recovered

Two cc of the distillate are placed in a test tube. 2 or 3 drops of a 19 per cent solution of potassium bichromate are added and then 2 cc of concentrated sulphuric



numbers are written on the inner surface of the ear with India ink, and, while the ink is still wet, the vibrating needle is run along the numbers, thus tatooing the pigment into the skin. This process is very rapid, a rabbit may be tatooed with a 3 digit number in about two minutes. The animal experiences little discomfort and no blood is drawn. The numbers put on by this method are permanent. No infections have been observed.

precipitation is a little more toward the right. In cerebral syphilis there is observed a change in the second to fourth tubes which does not exceed the third or fourth degree.

4 In meningitis the maximum change occurs between the fifth and tenth tubes. This change reaches high degrees in purulent meningitis and less high degrees in tuberculous meningitis.

The following general conclusions are drawn: a change in color reaching the third degree shows a pathologic fluid. The maximum precipitation occurs in Tubes 5 and 6 in syphilitic disease of the central nervous system and this is the syphilitic zone. The maximum precipitation occurs in higher dilutions in meningitis. He adds that the reaction is qualitative and that the characteristic curves in the different diseases of the central nervous system can be easily distinguished from each other.

**TISSUES** Quick Celloidin Method Rhamy B W Arch Path & Lab Med 5 282 1928

#### METHOD

- 1 Ten per cent formalin ten minutes
  - 2 Acetone (depending on size of tissue block), two changes from one half to two hours
  - 3 Acetone, absolute alcohol ether equal parts, from one half to two hours
  - 4 Acetone, 1 part, thin celloidin 3 parts, from one half to two hours
  - 5 Thick celloidin, twenty four hours
  - 6 Mount and harden in full strength denatured alcohol
- After step 3 the method may be varied thus if desired:
- 4 Absolute alcohol ether equal parts from one half to two hours
  - 5 Thin celloidin, from one half to two hours
  - 6 Thick celloidin, twenty four hours
  - 7 Mount and harden in 80 per cent alcohol

**SEX HORMONE** Clinical Data Obtained With the Female Sex Hormone Blood Test Frank R T and Goldberger M A J A M A 90 106 1928

The reaction consists in obtaining 40 cc of venous blood, pouring the unclotted blood in 60 cc of pure not denatured 95 per cent ethyl alcohol evaporating to dryness under reduced pressure triturating the residual powder finely, extracting twice with pure ether, again evaporating to dryness and emulsifying the ether soluble residue in 2 cc of sterile distilled water. The emulsion is injected in five divided doses, distributed over a period of ten hours into a castrated mouse, due precautions being taken to reduce the leakage from puncture holes to a minimum.

A vaginal smear obtained from the mouse in from thirty six to forty eight hours is recorded as

Negative 0 if there is a preponderance of leucocytes in the smear

Weak +2 if a large amount of nucleated epithelium is present but a few leucocytes persist

Threshold +3 if the smear contains an excess of epithelial cells no leucocytes

Strong +4 if the smear shows only nonnucleated squamous epithelial scales

Every positive reaction is controlled by daily vaginal spreads examined over a period of ten succeeding days in order to exclude regeneration of an overlooked fragment of ovary and consequently a mistake due to reestablishment of the spontaneous cyclic reaction.

The conclusions drawn from an investigation of the test in a number of varied conditions were that

In women with normal sex cycle more and more of the female sex hormone accumulates in the circulating blood until menstruation sets in.

With the onset of menstruation as well as with the beginning of pregnancy the hormone disappears from the circulating blood.

The hormone is found in great concentration in the menstrual as well as in the postpartum blood.

The hormone is found in the circulating blood from the twelfth to the fortieth week of gestation.

In functional bleeding a majority of cases show excessive ovarian activity

Functional overactivity may be demonstrated without excess bleeding (tension) and even in the presence of amenorrhea

Amenorrheas must be subdivided into (a) a grave type without cycle and into those with (b) subthreshold cycle, (c) self limited with impending menstruation, and (d) due to persistent corpus luteum The gravity of the condition depends on the type

Women who have never menstruated may nevertheless ovulate and their sex tract undergo cyclic changes

The test, when positive, permits of the determination of sex

Sterilities probably fall into two classes, the first with normal cycle, the second with depressed function In the first group other factors besides ovarian function must be taken into account

Death of the fetus after the twelfth week can be recognized by the blood test

#### **SMALLPOX** The Laboratory Diagnosis of Smallpox Virus Utilizing the rabbit, Defries, R D, and McKinnon, N E Am J Hyg 8 107, 1928

The reaction in the skin of the vaccine immunized rabbit following the intradermal injection of smallpox material may be useful in the diagnosis of smallpox, but is so variable, both in occurrence and in character, that accurate interpretation is often impossible

The corneal reaction is specific, but positive results have been found by the authors in only about one half of the tests when known smallpox material was used

The intradermal injection of smallpox material into normal rabbits evokes regularly a definite, specific lesion

The development and retrogression of the lesion produced by the intradermal injection of smallpox material into the normal rabbit differs markedly from the course of the lesion similarly produced in the vaccine immunized rabbit

The repeated intradermal injection of smallpox material into rabbits produces in them a definite immunity against vaccine virus

A laboratory test for smallpox virus is found in the development of the typical lesion following intradermal inoculation of smallpox material in the normal rabbit, contrasted with the response following a similar procedure in the vaccine immunized rabbit

#### **SPINAL FLUID** Further Data On the Boltz Test, Cady, L D Arch Neurol and Psychiat 18 565, 1927

The Boltz test is made by placing 1 cc of spinal fluid in a Wassermann test tube and adding 0.3 cc of acetic anhydride drop by drop The contents of the tube are then shaken, and 0.8 cc of concentrated sulphuric acid is added drop by drop The tube is again shaken and observed for about five minutes, when a blue pink or lilac color will develop in positive general paralytic fluids Boltz believes that the test is of diagnostic and prognostic significance in neurosyphilis

The Boltz test is a modification of the Liebermann cholesterol test, but these fragmentary observations fail to show that cholesterol is in any way responsible for the positive reaction The modified Boltz test has been used on 799 specimens of spinal fluid Positive reactions were found in from 79 to 94.6 per cent of patients with neurosyphilis when the test had not been rendered negative by treatment The modified test was positive in 42.4 per cent of 264 other nonsyphilitic neuropsychiatric patients It was found positive in 27.2 per cent of seventy seven patients with constitutional diseases This occurred for the most part in patients with arthritis of the spine The original test seems of some value in indicating the presence of abnormal processes in the meninges and the central nervous system, and promises to be of a value at least equal to other routine nonspecific chemical tests The modified test may have considerable value in the control of treatment of neurosyphilis These observations indicate that, whenever the test is used routinely, the original and the modified technique should be used on each specimen of spinal fluid examined

## REVIEWS

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Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building,  
Richmond, Va.

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### *Reagenzien und Nachrboden\**

THE collection of reagents is divided into those for qualitative tests, for quantitative tests, for physiologic chemistry and for microscopic technic. Then follows the culture media preserving and hardening fluids and miscellaneous formulas.

The authors are two German pharmacists and they apparently know little or nothing about modern American methods. For example there are more than 400 formulas for physiologic chemistry and yet there is nothing of modern blood chemistry or the micro methods. There are about 250 formula for culture media but neither E. M. B. agar nor Russell's Double Sugar Medium is given.

However it is a right complete collection of German formulas and includes many which sound right strange to us.

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### *Die Wasserstoffionenmessung†*

THIS is a small book of only 140 pages which attempts to give the essentials of the determination of hydrogen ion concentration in the simplest possible manner so that those without special training in physical chemistry or mathematics can understand it. After preliminary chapters on solutions ionization and buffers the author explains the process of determining the hydrogen ion concentration both by the use of indicators and by electrical methods for the worker who has no other help than this little book. Clark and Lubs indicators and other American work are given recognition.

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### *Principles of Public Health Engineering‡*

THIS volume is the outgrowth of a course in public health engineering given by the author at Columbia University and is intended for the medical health officer without engineering training and for the sanitary engineer without knowledge of public health principles. "In brief the book attempts to furnish the public health background to the conventional sanitary engineering course and an engineering viewpoint to the medically trained man doing public health work."

In this aim the book is commendably successful and serves a useful purpose. For those interested in the subject there are lists of collateral reference works at the end of each chapter.

The book can be recommended to those to whom it is addressed.

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*Reagenzien und Nachrboden* (Reagents and Culture Media—A Collection of the Most Important and Reliable Formulas for the Laboratory) By Boehm and Dietrich Urban and Schwaizenberg. Berlin.

*Die Wasserstoffionenmessung* (The Measurement of Hydrogen Ion Concentration) By Günther Lehmann. Published by Johann Ambrosius Barth, Leipzig.

*The Principles of Public Health Engineering* By I. F. Phelps. Professor of Sanitary Science, Columbia University. Cloth, 16 pages, 35 figures. The Macmillan Co. New York.

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NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in the foregoing pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

## *Growing Up\**

MODERN educators and modern parents are agreed upon the wisdom, the benefits, and the necessity of imparting to children a correct knowledge of sex life. It is one thing, however, to have knowledge and another to be able to impart it, and gifted, indeed, are they who can speak to children on these matters in a tactful, understandable, and inoffensive way.

Because of this inherent difficulty there is a tendency to pass on this responsibility and for each, the parent, the teacher, the doctor and so on, to evade this duty, in large measure for lack of a satisfactory way to go about it.

Of all the books which have been written, there are none so commendable, none so valuable, and none so entirely satisfactory as this one by Karl de Schweinitz.

There is nothing in it which smacks of "now children", there is nothing to arouse suspicion that something is being suppressed, nothing to awaken unsatisfied curiosity, nothing to cause embarrassment to child or parent, nothing incomprehensible to a child's mind. The book is marvelously natural, entirely matter of fact, of absorbing interest and, without, accurate and complete. There is no glossing over, no omissions, the book admits of no criticism for, and this, indeed, is its main virtue, that without the slightest suggestion of the teacher, without any portentous solemnity, it leaves the child reader undisturbed in the understanding that mating and birth are natural, seemingly, and good things concerning which there should be no false cloak of shame or prudery.

To the parent and teacher, and to the harassed doctor consulted upon how such teaching should be done, this book will be of inestimable value.

There is only one regret, that there is no way in which it could be placed in the hands of every parent and every child.

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## *Medical Bacteriology†*

THIS book is intended primarily for the medical student and practitioner, its purpose being to present simply in a practical way the accepted relation of bacteria to medicine.

It is divided into two parts. Part I, Descriptive Bacteriology, presents in a simple yet relatively comprehensive manner, a discussion of the properties of bacteria in relation to disease, the ordinary methods of cultivation, staining, etc., available for their study and identification and in various serologic and pathogenicity tests. There is a short but useful chapter on common contaminating organisms following which the bacteria of ordinary pathogenicity are described.

Fungi, filterable viruses, protozoal diseases and helminth infections are next described. There is a short but practical chapter on bacterial preparations: vaccines, sera, etc.

In Part II, Applied Bacteriology, the uses of bacterial preparations are discussed. Because the bacteriologic examination may be, and, as the practical experience of any pathologist will testify, often is, ruined by a faulty collection of the specimen, a chapter is devoted to this.

Then follows a discussion of laboratory aids in the diagnosis and treatment of various diseases which are arranged in alphabetical order. The final chapter discusses succinctly the bacteriology of water, milk and food.

This little book should be eminently useful to those to whom it is addressed.

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## *Anthelmintics and Their Uses‡*

IN THE preface to this distinctly useful book, the authors comment very pertinently upon the fact that helminths form a separate and distinct group of organisms and that the drugs used to combat them are also a distinct group since they are seldom used for other purposes.

\**Growing Up*. By Karl de Schweinitz. Cloth. 111 pages. 30 illustrations. The Macmillan Co. New York.

†*Medical Bacteriology Including Elementary Bacteriology*. By L. H. Whitby. Cloth. 320 pages. 75 illustrations. J. and A. Churchill. London.

‡*Anthelmintics and Their Uses in Medical and Veterinary Practice*. By R. N. Chopra, M.D., Professor Pharmacology, Calcutta School of Tropical Medicine and Hygiene and A. C. Chandler, Ph.D., Professor of Biology, Pice Institute, Houston, Texas. Cloth. 291 pages. Williams and Wilkins Co. Baltimore, Md.

Because of these facts because the organisms differ in their reaction to drugs because many of the details concerning anthelmintics and their uses appear in relatively inaccessible places, and because by virtue of the structural and physiologic diversity of helminths there can be no universal anthelmintic this book has been written.

It is divided into three general sections in the first of which the general considerations applicable to the subject are discussed together with a discussion of the correlation between chemical composition and anthelmintic action.

Section II is devoted to a discussion of anthelmintics acting on parasites in the gut and Section III considers those used against cutaneous parasitic infections.

The literature of the subject has been freely consulted and evaluated in the light of the authors' experience.

The book, stated to be the first of its kind, should fill a very useful place in the practitioner's library.

### *A Textbook of Biologic Assays\**

THIS the second edition of a well known and extremely practical manual has been so extensively revised as to live in practically rewritten. It may be regarded as an authority in its field and highly commended to those engaged or interested in pharmaceutical assay or drug standardization.

### *Laboratory Manual of Physiological Chemistry†*

THIS manual is intended primarily for the teaching of students of biochemistry and presents in nine chapters a well ordered series of exercises including the accepted modern methods especially quantitative of physical chemistry. It is interleaved for the insertion of notes and should prove acceptable to both teacher and student.

### *Clinical Laboratory Procedures‡*

THIS is essentially a compendium of the various methods used in the author's laboratories the book being interleaved for the convenience of the user.

### *The Determination of Hydrogen Ions§*

NEITHER this book nor its author requires an introduction. Dr. Clark's pioneer studies in the study of hydrogen ion determination and his relation to the development of this field of biologic chemistry are sufficient in themselves to assure the authenticity and authority of this volume.

The present edition embodies a comprehensive review of all the advances in technique and all the applications of hydrogen ion determination to biologic studies in health and disease.

Despite the complexities of the subject the style is exceedingly clear and at the same time happy and scholarly. It is a volume to be owned and read with pleasure as well as with immeasurable profit.

It is a welcome innovation indeed to read a volume the nature of whose subject would lead the reader to apprehend a ponderous style enlivened by chapter mottoes as it were both grave and gay and even from Alice in Wonderland but to the point nevertheless.

Dr. Clark and the publishers are to be congratulated and the reader as well that such a book awaits his perusal and study.

\* *A Textbook of Biologic Assays*. P. P. C. Pittenger, Ph.D., etc. Instructor in Biologic Assays, Philadelphia College of Pharmacy, etc. Second edition. Cloth, 312 pages, 15 illustrations. P. Blakiston's Son & Co., Philadelphia.

† *Laboratory Manual of Physiological Chemistry*. By M. Rodan, A. Rodan, Prof. of Biological Chemistry and M. S. Fay, Adjunct Prof. of Biological Chemistry, University of Texas. Cloth, 34 pages, 9 figures. John Wiley & Sons, Inc., New York.

‡ *Clinical Laboratory Procedures*. By G. L. Roblenburg, M.D., Director of Laboratory, Lenox Hill Hospital. Cloth, 66 pages. The Macmillan Co., New York.

§ *The Determination of Hydrogen Ion*. By H. M. Clark, Professor of Physiological Chemistry, Johns Hopkins University. Ed. Cloth, 177 pages, numerous tables. Williams and Wilkins Co., Baltimore, Md.

# The Journal of Laboratory and Clinical Medicine

VOL XIV

ST LOUIS, MO, JUNE, 1929

No 9

Editor WARREN T VAUGHAN, M D

Richmond, Va

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Official Organ of the American Society of Clinical Pathologists and the  
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## EDITORIALS

### The Sanitary Significance of Lactose-Fermenting Bacteria Not Belonging to the B Coli Group

THE determination of the potability of water, a matter of great sanitary importance, has always been the subject of some confusion in the lay mind. There are many, for example, who believe implicitly that all that is necessary is a single bacteriologic study, failing to appreciate that such an examination applies only to the water at the time of examination, that it offers no absolution for the sins of the past and no guarantee for the future and that without interpretation in terms of a sanitary survey, it is but a reed to lean upon.

There is a popular idea, also, that running water, if not always pure, will purify itself in its course, an idea which investigations of the U S Public Health (Bull 173) have shown to be quite erroneous.

These fallacious conceptions at times render the interpretation to the public of bacteriologic water examinations a matter of difficulty, especially as the subject itself has inherent difficulties demanding careful consideration.

In a general way, without entering into a discussion of the minutiae of the subject, bacteriologic examinations are largely concerned with the detection of *B. coli*, and much interest, therefore, attaches to those organisms, which may be a source of confusion.

Believing that the sanitary importance of such bacteria, lactose fermenters not belonging to the colon group has been somewhat overlooked Greer<sup>1</sup> and his collaborators have recently restudied the question in some detail based upon examinations of the Chicago water supply.

The most common lactose fermenting bacteria encountered in water are *Cl. Welchii*, *B. aerosporus*, *Streptococcus fecalis* and members of the mucosus capsulatus group.

In the Chicago water supply these and related bacteria were shown to have a seasonal variation and to vary in raw and treated water, so much so as to render valueless, during many months of the year the forty eight hour presumptive test of treated water as an index of *B. coli*. In Chicago therefore, the presumptive test must be carried to completion to avoid error during the greater part of the year.

This fact is of practical importance and brings up a number of questions in connection with the use of lactose broth for the isolation of any single organism.

A review of the literature shows that *B. coli* is outgrown and inhibited in culture media by several bacteria found in water. The experimental work of Greer and his associates shows that a) *B. coli* may be outgrown by *S. fecalis*, *Cl. Welchii* and *Ps. pyocyaneus* in forty eight hours b) *B. aerosporus* is outgrown by all these organisms in both twenty four and forty eight hours, c) If *B. coli* is present in these mixtures, it can usually be isolated in twenty four hours from tubes showing gas within this time d) Combinations of organisms found in water may give rise to gas production which is not due to the presence of *B. coli*.

A study of the literature reveals that only two of the lactose fermenting organisms other than *B. coli*, *Cl. Welchii* and the Friedlander group, are commonly associated with pathologic conditions in man. *Ps. pyocyaneus* may or may not be pathogenic when ingested. Greer and his coworkers found it usually associated with *B. coli*, and believe that its potential properties warrant the condemnation of a water from which it is isolated.

The question of the survival of microorganisms in water is of marked importance.

Water is not a satisfactory menstruum for the survival of intestinal bacteria and the hydrogen ion concentration, temperature, sunlight and sedimentation all play an important role in determining the survival of intestinal bacteria in water.

*B. coli* usually survives longer than *B. typhosus*. *S. fecalis* perishes rapidly, while the evidence suggests that spore formers survive the longest. Due to



the variety of factors influencing the results, definite time tables of survival are difficult to compile. Each water with the concomitant circumstances is, to some extent, a law unto itself.

The results of this study are of interest in connection with the technique of bacteriologic water examination, the present tendency of which has been toward the use of selective media and methods adapted to the detection of *B. coli* to the exclusion of other bacteria.

The work of Greer and his associates leads them to believe that the best combination of mediums and methods include lactose broth for the presumptive test, eosin methylene blue for the confirmatory test, and lactose broth and agar slants for the completed test.

Their conclusions may be thus summarized:

Besides *B. coli*, *S. fecalis*, *Cl. Welchii*, and *Ps. pyocyaneus* may all be of sanitary importance in water examination, *S. fecalis* probably having as much sanitary significance as *B. coli*, while *Cl. Welchii* and *Ps. pyocyaneus* are of less significance, and *B. aerospirius* of no significance.

While not suggesting that any of these organisms are any better indexes of fecal pollution than the fecal type of *B. coli*, they stress the fact that there are lactose-fermenting bacteria in water other than the colon-aerogenes group, some of which have sanitary significance and cannot be ignored.

They suggest, therefore, that it may well repay routine control laboratories to discover what lactose-fermenting organisms other than *B. coli* are present in their water supplies, and to study and determine the potentialities of the types of pollution present, especially as such investigation may reveal the source of false negative and positive results encountered in the routine examination for *B. coli*.

#### REFERENCES

- 1 Greer, F. E. Sanitary Significance of Lactose Fermenting Bacteria Not Belonging to the *B. Coli* Group. I. Groups Reported in the Literature and Isolated from Water in Chicago, *J. Infect. Dis.* 42 No. 5, 501, 1928.
- 2 Greer, F. E. II. Number of Lactose Fermenting Organisms Found in Chicago Sewage & Chicago Water Supply, *Ibid.* 42 No. 5, 514, 1928.
- 3 Greer, F. E., and Nyhan, F. V. III. Bacterial Associations in Cultures Containing Lactose Fermenting Bacteria, *Ibid.* 42 No. 6, 525, 1928.
- 4 Greer, F. E., Jounev, F. O., and Nyhan, F. V. IV. Pathogenicity, *Ibid.* 42 No. 6, 537, 1928.
- 5 Greer, F. E. V. Factors Influencing the Survival of Microorganisms in Water, *Ibid.* 42 No. 6, 545, 1928.
- 6 Greer, F. E. VI. Sanitary Considerations, *Ibid.* 42 No. 6, 551, 1928.
- 7 Greer, F. E., Noble, R. E., Nyhan, F. B., and O'Neil, A. E. VII. Mediums and Methods, *Ibid.* 42 No. 6, 556, 1928.
- 8 Greer, F. E., and Noble, R. E. VIII. Conclusions, *Ibid.* 42 No. 6, 568, 1928.

—R A K

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## American Society of Clinical Pathologists

### Program

### (Of the Eighth Annual Convention)

Portland Oregon

July 5 6 and 8 1929

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Rochester, Minnesota	Newark, N J
DR JOHN A KOLMER - - - - 1924 5	DR A H SANFORD - - - - 1927 8
Philadelphia, Pennsylvania	Rochester, Minnesota

FRIDAY, JULY 5, 1929, 9 A M

Call to Order

Short Business Session

**Scientific Program**

- The Effect of the Presence of Bile on the Agglutination Reaction By Ruth Gilbert, M D, and Marian B Coleman, BS, Albany, N Y
- Tularemia (Francis' Disease) A Report of Ten New Cases with an Analysis of Sixty three Cases Occurring in Dayton, Ohio By Walter M Simpson, M D, Dayton, Ohio
- The Routine Use of the Photoelectric Hemoglobinometer By A H Sanford, M D, and Charles Sheard, Ph D, Rochester, Minn
- Polycythemia (Patent Foramen Ovale) By Herman Spitz, M D, Nashville, Tenn
- Acute Diffuse Myelitis Following Intravenous Injection of Neoparsphenamine By Ernest Scott, M D, and H L Reinhart, M D, Columbus, Ohio
- Spontaneous Meningeal Hemorrhage By Frederick H Lamb, M D, Davenport, Iowa

FRIDAY, JULY 5, 1929, 2 P M

**Symposium on Undulant Fever**

- Undulant Fever in Man A Clinical Analysis of Thirty three Cases By A S Giordano, M D, and R L Sensenich, M D, South Bend, Indiana
- Discussion on Undulant Fever By Walter M Simpson, M D, Dayton, Ohio
- The Pathogenicity for Monkeys of Brucella Abortus By Merrill J King, M D, Mount MacGregor, New York (By invitation)
- The Etiology and Diagnosis of Undulant Fever in the United States By Charles M Carpenter, M D, and Ruth Book, Ph D, Ithaca, New York (By invitation)
- Some Observations on the Agglutination of B Abortus By Frank B Lynch, Jr, M D, and Annette M Callan, Philadelphia, Pa
- Notes on the Bacteriology of the Brucella Group By K J Meyer, M D, San Francisco, Calif, to be read by J C Geiger, M D, San Francisco, California (By invitation)

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**Round Table Discussion**

- Virtuosity in Clinical Pathology By Philip Hildkowitz, M D, Denver, Colo
- Problems By W G Gamble, Jr, M D, Chicago, Illinois
- The State Laboratory Problem By Edward F Cooke, M D, Houston, Texas
- The Hospital Situation
- A Economics By Herman Spitz, M D, Nashville, Tennessee
  - B Scientific By Robert A Keilty, M D, Washington, D C
  - C Statistical By Philip B Matz, M D, Washington, D C
  - D Relation to the American College of Surgeons By J J Moore, M D, Chicago, Ill
- Postmortems By Herbert R Mills, M D, Tampa, Fla
- Is the Cost of Laboratory Work Too High? By Robert F E Stier, M D, Spokane, Wash
- The Clinical Pathologist in the Rural Hospital By C W Mavnyard, M D, Pueblo, Colo

## SATURDAY, JULY 6 1929 9 A M

Gingivitis V The Character of the Exudate in Gingivitis By Robert A Kellv M D,  
Washington, D C

The Tuberculous Cavity By Alfred Blumberg M D, Oteen N C

Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of Acid  
fast Non Pathogens on Different Mediums with Their Reaction to Chemical Reagents  
By H Y Corper M D and Geo Uvel Ph D Denver Colo

A Recently Isolated Bacillus of the Hemophilus Group By Frank W Hartman M D and  
Edna Jackson M S Detroit Mich

Immunological Specificity of Green Producing Streptococci Having Elective Localizing Power  
as Isolated in Various Diseases By E C Rosenow M D, Rochester Minn

Milk Borne Rabies By E R Mudge M D Denver Colo

Observations on Intestinal Protozoosis By Rawson J Pickard M D, San Diego Calif

## SATURDAY JULY 6 1929 2 P M

Pathology of the Reticulo Endothelial System By Zera E Bohn M D San Francisco, Calif  
Reticuloocytes Their Identification and Significance By C L Spohr, M D and Alice Bastine  
Columbus Ohio

Improved Colorimetric Procedures for the Quantitative Estimation of the Proteins of the Cere-  
brospinal Fluid By Philip B Matz M D and Nathan Novick Washington D C

Quantitative Microscopic Urinalysis By William G Exton M D Newark N J  
New Quantitative Clinical Methods for the Junior Scopometer

- |                           |                               |
|---------------------------|-------------------------------|
| 1 Protein in Urine        | 6 Sugar in Blood              |
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| 4 Globulin in Urine       | 9 Creatinine in Urine         |
| 5 Sugar in Urine          | 10 Sulphur Partition in Urine |

By William G Exton M D Anton R Rose Ph.D, Fred Schattner Ph D and P V  
Wells D Sc, Newark, N J (To be read by Title)

Embryonal Carcinoma of the Testicle By L W Larson M D, Bismarck, N D

Malignant Tumors of the Testicle By O A Brines M D, Detroit Michigan

## SATURDAY JULY 6 1929, 7 P M

## Annual Banquet

Presidential Address By Dr Frank W Hartman Detroit Mich

Address By Dr Richard B Dillehunt Dean of the Medical School of the University of  
Oregon, Portland Ore

Address By Dr Cyrus C Sturgis, Professor of Medicine at the University of Michigan,  
Ann Arbor Mich

Presentation of the Ward Burdick Research Award

## MONDAY, JULY 8 1929, 9 A M TO 12 AND 2 P M TO 5 P M

## Business Session

Call to Order

Reading of Minutes

Unfinished Business

Reports of Committees

Executive Committee—Dr A H Sanford, Chairman Rochester, Minn

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Report of Board of Censors—Election of New Members

New Business

Report of Nominating Committee—Nomination of Officers

Election of Officers

Induction of Officers

Adjournment

### Scientific Exhibits

Apparatus By B W Rhamy, M.D, Fort Wayne, Ind

Mounted Specimens of Pathology By C H Manlove, M.D, Portland, Ore

Razor Section Method By B T Terry, M.D, Rochester, Minn

The Problem of Gingivitis By Robert A Keilty, M.D, Washington, D.C

Scientific Exhibit By Alfred Blumberg, M.D, Oteen, N.C

Specimens and Pictures of a Case of Occidiodal Granuloma By D Schuyler Pulford, M.D, Woodland, Calif

### Personals

In the Bulletin of the American Hospital Association for May, 1929 we read that Dr J J Moore of Chicago spoke before the Indiana Hospital Association in Indianapolis, April 11th, on the financial phase of the hospital laboratory

That the specialty of clinical pathology is far from foundering on the rocks is evidenced by the fact that a number of our men are taking summer voyages to Europe Dr Frederic F Sondern New York City, is making his annual pilgrimage abroad Dr Alvin G Foord of Buffalo, N.Y., is leaving for the Continent the last of May Dr A H Schade, Toledo, Ohio, plans a trip for the fall

Dr W G Gamble, Jr, of Chicago is making a strenuous effort among the members of the American Society of Clinical Pathologists to charter a special car from Chicago to the Portland Convention

Dr William J Deadman, President of the Hamilton Medical Society, will, in his official capacity, act as host to the Ontario Medical Association Convention during the latter part of May

The increasing importance of the Laboratory as an aid to diagnosis is demonstrated in the comparatively frequent papers on the subject at various state meetings At the meeting of the Ontario Medical Association Dr S H McKee of Montreal will speak on the "Relation of the Clinical Laboratory to Clinical Ophthalmology"

# *The Journal of Laboratory and Clinical Medicine*

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VOL XIV

ST LOUIS MO JULY 1929

No 10

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## *CLINICAL AND EXPERIMENTAL*

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### A BACTERIOLOGIC STUDY OF FIVE HUNDRED SIXTY SEVEN POSTMORTEM EXAMINATIONS\*

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HENRY F HUNT M D AND ETHEL BARROW M B, Ch B  
*Fellows in Pathology The Mayo Foundation*

LUTHER THOMPSON Ph D  
*Section on Clinical Pathology The Mayo Clinic*

AND GEORGE W WALDRON M B  
*Technical Assistant*

ROCHESTER MINNESOTA

---

THE value of most postmortem examinations is enhanced tremendously if a careful bacteriologic study is made on the fluids and organs of the body in many instances the entire solution as to the cause of the patient's illness and death can only be reached by such bacteriologic research

In the presence of abscesses peritonitis pneumonia etc if a specific organism is found the diagnosis is complete The finding of Actinomyces Streptothrix and amebae has a similar significance The work of the bacteriologist is even more necessary when for various reasons proper studies cannot be made before death Hence the cause of death which may have been obscure can either be definitely determined or corroborated

Judging from the literature postmortem bacteriology has received little attention and moreover is receiving even less in recent years Many problems present themselves in this phase of bacteriology and certain workers in this field consider the results so conflicting as to be unsatisfactory The questions confronting one in the interpretation of the results obtained in a study of this problem are Is there a terminal or agonal invasion of the blood stream

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Received for publication September 5 1928

Work done in Sections on Clinical Pathology and Pathologic Anatomy The Mayo Clinic  
Submitted for publication September 1928



and tissues of the body by bacteria already present within the gastrointestinal and pulmonary tracts? Is this terminal invasion of frequent occurrence? Does it occur in one type of case more often than in another? If such invasion does occur, what are the most probable foci of origin and what part do they play in the death of the patient? Do bacteria migrate from foci or from the gastrointestinal or respiratory tracts after death and if so how soon? There are as many variations to the question of dissemination of bacteria after death as to the ones regarding terminal or agonal invasion of bacteria.

Because of the foregoing difficulties the few workers in the field who have reported the results of their efforts have reached various conclusions. Some support the reliability of the results, others believe that invasion of the blood and organs either before or after death renders the results valueless.

Many of the earlier students of this subject considered that postmortem cultures were of little value. Wurtz and Herman, Hauser and Birch-Hirschfeld found *Bacillus coli communis* to be a common invader of the blood stream and internal organs after death. They concluded that dissemination of bacteria from certain organs particularly from the gastrointestinal tract, takes place soon after death, and because of this the results obtained from post-mortem bacteriology were unreliable. It is noteworthy that in none of the cases which they mentioned was necropsy performed less than ten hours after death.

Friedette, although he admits the possibility of invasion after death, believes that cultural results obtained a few hours after death are fairly reliable in demonstrating the presence of organisms at the time of death.

Canavan and Southard, Landstrum and Austerlitz, and Monod and Micaigne concluded that the heart's blood, liver and spleen were seldom the seat of terminal invasion by bacteria of the intestinal tract, and that the bacteria found were of intravital occurrence, at least during the few hours immediately after death.

Flexner has pointed out that secondary or terminal infections are relatively common, particularly in chronic diseases.

Low, Canon, Achard and Phulpin, and Gwyn and Harris, all agree that postmortem cultures should be made as a routine.

Giordano and Barnes, in 1922, thoroughly reviewed the literature and reported their results from a careful bacteriologic study of 213 postmortem examinations. They concluded that the bacteriologic changes noted may strengthen, illuminate, or sharply modify the cause of death, as revealed by clinical diagnosis. They admit the possibility of terminal invasion. Its occurrence must not be dismissed lightly as it may be the most important contributory factor to the cause of death.

Our results were obtained by working on the problem consecutively and independently for two and a half years. Each worker used a similar technique. Cultures were taken when possible from the heart's blood, liver and spleen, and from other organs when indicated. Accumulations of fluid and purulent material were also cultured. Blood from the heart was obtained usually from the pulmonary artery by means of a sterile syringe and needle. The surface of the artery was always sealed either by means of a heated spatula or a projected flame. The surface of the organs to be cultured was sealed in the same way and

the material was obtained through this sterile field by means of a special glass pipette or a metal loop. Pus and other fluids were obtained by aspiration or swabs. The utmost care was observed to eliminate contamination. Rosenow's dextrose brain broth and the ordinary blood agar plates were inoculated as a routine with the material obtained for culture. As occasion demanded special mediums were inoculated. All mediums were inoculated within one hour after the material to be cultured had been obtained. For comparison and control direct smears were made from inflammatory surfaces and from the contents of abscesses. These smears were then stained and examined for organisms.

The value of our work was enhanced by the fact that the bodies had not been subjected to refrigeration and that the cultures were obtained within one to twelve hours after death most of them within three hours. Table I shows that the ratio of positive to negative cultures more or less corresponds throughout. The three positive cultures of the third worker and the one positive culture of the first worker taken twelve hours after death were obtained from cases in which there was extensive infection prior to death. One case was that of septicaemia and from the extent of the infective lesions in the other cases terminal septicaemia might have been present. We concluded therefore, that at least twelve hours may elapse between death and postmortem examination without affecting the reliability of bacteriologic studies. This agrees with the view of Fredette who believes that cultures obtained a few hours after death are fairly reliable in demonstrating organisms present at the time of death. Giordano and Barnes also concluded that invasion of the blood stream rarely occurred and that sustained progressive increase in positive results at successive hours after death did not occur. In their series they covered a period of twenty three hours after death.

TABLE I  
POSITIVE AND NEGATIVE CULTURES OF HEART'S BLOOD TIME OBTAINED AFTER DEATH

HOURS	FIRST WORKER		SECOND WORKER		THIRD WORKER		FOURTH WORKER	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
1	22	41	11	18	13	40		11
2	21		17	21	8	40	5	10
3	6	20	12	1	1		1	9
4	2	13		4		5	2	4
5		3	3	4	6	4		2
6		3	1			8	1	1
7	2		0	1	2	2		
8	1	2			2	10		
9	1			1	2	2		
10	1	1	2	2		1		
11		1				1		
12	1				3			
Total	63	138	33	70	4	140	1	40

On the other hand Birch Hirschfeld cultured the internal organs and heart's blood of twenty cadavers ten hours after death and found *Bacillus coli* in all of them. Wuitz and Heermann also found the bacillus in the liver, spleen and kidneys of cadavers cultured twenty four to thirty six hours after death.

The total number of blood cultures made at postmortem and the total number which were positive and the percentage of positives are shown in

Table II These data, when compared with those of other workers are low except those of the second worker, in this group there was a much higher percentage of cases in which there were definite foci

The percentage of positive blood cultures obtained after death by other investigators is as follows Friedette 35, Simmonds 48, Gładwohl 44, Canavan and Southard 44, Richey and Goehring 29, and Giordano and Barnes 38.8 Strauch, in 2000 cultures found 1001 cultures sterile The cultures were obtained from the heart's blood at necropsy on an average of ten to fifteen hours after death A marked variation may also be noted in the percentage of positive cultures obtained in our series It can be easily understood that the type of cases dealt with by each worker must have a great deal of influence on the results obtained For instance, a preponderance of cases in which there are infectious foci will raise the percentage of positive blood cultures In only one case in our combined series did we find a positive blood culture without definite foci

We considered that foci of infection were present in all cases in which the lesion was demonstrated pathologically, such as cases of peritonitis, abscess, meningitis, pneumonia with abscess formations, and ulcerative colitis By comparing the percentage of cases with definite foci with the percentage of positive blood culture (Table II) a uniformity of results may be seen except in

TABLE II  
CASES WITH POSITIVE BLOOD CULTURES AND DEFINITE FOCI

	BLOOD CULTURES			DEFINITE FOCI OF INFECTION		DEFINITE FOCI OF INFECTION WITH POSITIVE BLOOD CULTURES	
	NUMBER	POSITIVE	PER CENT POSITIVE	CASES	PER CENT	CASES	PER CENT
First worker	201	63	31.3	92	45.8	62	67.4
Second worker	123	53	43.7	90	73.2	53	58.9
Third worker	185	45	24.3	88	47.6	45	51.1
Fourth worker	58	12	20.7	31	53.4	12	38.8

the case of the first worker and the fourth worker The discrepancy in the former group can be explained by the fact that, although the percentage of cases with definite foci was small in comparison with the others, yet the percentage of positive blood cultures was high, thus raising the total percentage of positive blood cultures In the latter group the percentage of cases with definite foci was high with a low percentage of positive blood cultures, thus decreasing the total percentage of positive blood cultures A study of the necropsy records showed the variation of percentage of positive blood cultures in cases with definite foci to be dependent on the severity and extent of the lesion present

A list of simultaneous cultures of heart's blood, spleen and liver and of positive cultures was made in an attempt to determine whether cultures from any one organ indicated better than those of another the presence of bacteriemia As this list only included cases in which all three organs had been cultured simultaneously we were able to study the behavior of each organ to the bacteria present in the same case Our data seemed to show that the

blood was a better indicator of bacteriemia than either the spleen or the liver.

Because of the generous blood supply of both liver and spleen it might be expected that if a positive culture was obtained from the blood itself, the organs also should contain similar bacteria. We found however that this did not occur (Table III). Whether this is due to inhibitory or bacterioidal influences of these organs we are unable to say. Cultures from the liver were more often positive than those from the spleen. This may mean that the fluids of the spleen are more bacterioidal than those of the liver, or that the liver is more liable to involvement through its portal circulation in cases of peritonitis or gastrointestinal lesions.

TABLE III

A COMPARISON OF CULTURES OF HEART'S BLOOD, SPLEEN AND LIVER MADE SIMULTANEOUSLY

	CULTURES OF HEART'S BLOOD SPLEEN AND LIVER	HEART'S BLOOD SPLEEN AND LIVER POSITIVE	HEART'S BLOOD ALONE POS- ITIVE	SPLEEN ALONE POSITIVE	LIVER ALONE POSITIVE
First worker	13	31	6	4	2
Second worker	83	11	16	1	6
Third worker	10				2
Fourth worker	41	3	2		

Our results are in direct opposition to those of Giordano and Barnes who in comparing cultures of the blood and spleen made simultaneously concluded that the spleen serves as well if not better than the heart's blood for determining terminal bacteriemia.

A careful survey was made of all cases which were considered after post mortem examination to be of nonbacterial origin, such as brain tumors, diseases of the thyroid gland, cardiovascular diseases and blood dyscrasias. The cases of this type studied by each worker were 109, 33, 97, and 27 respectively. In only one of these was a positive culture obtained from the heart's blood, liver or spleen. The time that the cultures were made varied between one and twelve hours after death. Postmortem invasion had not occurred. In the one case in which positive blood culture was obtained the diagnosis before death and at necropsy was acute lymphatic leucemia. At necropsy hemolytic streptococcus was found in the heart's blood, liver and spleen but infectious foci were not demonstrated. Necropsy was performed ten hours after death. The patient for four months before death had been subject to colds and his recent illness was of two weeks' duration in which time he ran a temperature as high as 104°. Septicemia was not suspected and blood cultures were not obtained before death. It cannot be proved that septicemia was present but considering the course of the terminal illness and the bacteriologic changes noted at postmortem examination this might have been the case.

#### SUMMARY AND CONCLUSIONS

1. This paper is based on a study of 567 postmortem examinations. The bacteriologic work was carried out by four persons working independently but consecutively.

2 The cultures were taken from unincubated bodies one to twelve hours after death

3 It was found that the lapse of twelve hours after death did not affect the results

4 The percentage of positive postmortem blood cultures obtained by each worker was 31, 43.7, 24, and 20.7 respectively

5 In all but one case in which cultures of blood were positive definite foci of infection were demonstrable

6 It was found that the number and type of cases in which infectious foci were definitely present was in marked relationship to the percentage of positive blood cultures

7 By comparison of simultaneous cultures from the heart's blood, liver and spleen it was concluded that the blood is a better indicator of terminal bacteremia than either the liver or spleen

8 In all but one case of nonbacterial diseases, the cultures were entirely negative

9 From the uniformity of results, it is believed that postmortem bacteriology is reliable and, if carried out carefully and as a routine, often leads to the solution of the exact cause of death

#### REFERENCES

- 1 Achard, C., and Phulpin, E. Contribution à l'étude de l'enrichissement des organes par les microbes pendant l'agonie et après la mort, *Arch de med exper et d'anat path* 7 25-47, 1895
- 2 Birch Hirschfeld. Quoted by Wurtz and Herman
- 3 Cunniff, M. M., and Southard, E. F. The Significance of Bacterial Cultivated from the Human Cadaver, a Second Series of One Hundred Cases of Mental Disease, With Blood and Cerebrospinal Fluid Cultures and Clinical and Histological Correlations, *J Med Res* 31 339-365, 1914-1915
- 4 Cunniff. Quoted by Simmonds
- 5 Flexner, Simon. A Statistical and Experimental Study of Terminal Infections, *J Exper Med* 1 559-576, 1896
- 6 Fredette, J. W. Bacteremias in the Agonal Period, *J Lab & Clin Med* 2 180-188, 1916-1917
- 7 Giordano, A. S., and Barnes, A. R. Studies in Postmortem Bacteriology, Value and Importance of Cultures Made Postmortem, *J Lab & Clin Med* 7 535-546, 1921-1922
- 8 Gradwohl, R. B. H. Importance de l'examen bactériologique pratique sur les cadavres, *Ann de l'Inst Pasteur* 18 767-773, 1904
- 9 Gunn, N. B., and Harris, N. M. A Comparison Between the Results of Blood Cultures Taken During Life and After Death, *J Infect Dis* 2 514-528, 1905
- 10 Hauser, A. Quoted by Simmonds
- 11 Lundstrum and Austerlitz. Quoted by Simmonds
- 12 Low. Quoted by Simmonds
- 13 Monod, C. and Micrigne. Contribution à l'étude des infections par streptocoques, *Rev de chir* 14 81-106, 1894
- 14 Richet, D. J., and Goehring, Carl. Studies on Bacteremias in the Agonal Period, *J Med Res* 38 421-447, 1918
- 15 Simmonds, M. Über bakteriologische Blutuntersuchungen an der Leiche, *Arch f path Anat* 175 415-442, 1904
- 16 Struich. Quoted by Fredette
- 17 Wurtz, R., and Herman, M. De la presence frequente du bacterium coli commune dans les cadavres, *Arch de med exper, et d'anat path* 3 734-745, 1891

# THE BIOLOGIC CHEMISTRY OF SKIN DISEASES AND SYPHILIS\*

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DURING the early ages the association of chemistry and medicine was illustrated by the efforts of the alchemist in his endeavor to prepare the elixir of life. On more stable foundation in the early part of the sixteenth century appeared that eccentric genius Paracelsus who looked upon medicine from the point of view of chemistry. Later there is found van Helmont the originator of chemical physiology. Many other chemist physicians occupied chairs in the universities and contributed in a large measure to medicine and chemistry. The pioneer and epoch results came in 1828 when Woechler and later Liebig removed the barrier which was believed to separate organic and inorganic compounds, the chemistry of living things from the inert. Here the beginning of the study of life and its processes became related to the chemistry of so called inert matter. For instance Sir William Prout discovered the presence of hydrochloric acid in gastric contents thus placing a vital relationship with the chemistry, physiology and pathology of digestion. The work of Pasteur is well known to all of us. The association of gout and uric acid likewise was also one of the early achievements. Thus it is seen that the chemical examination of the blood of living beings afforded methods of diagnosis which in turn has developed research on new substances and the presentation of methods of greater accuracy. In relation to the report to be presented in this paper the investigations of Frederick William Pavy who devoted much of his time to the study of diabetes never saw the fulfillment of his vision of his research the discovery of insulin. He however so carefully described cyclic albuminuria that a rich heritage for later investigators was placed at their disposal. In this case again it appeared that too much time was being spent on an incurable malady yet time, energy and vision conquered the apparent insurmountable obstacle and the time was not wasted. Thus in a wider sense and in a wider field the study of living cells and processes has given us a host of great biochemists such as Pasteur, Wurtz, Hoppe Seiler, Abderhalden, Schmidt, Emil Fischer, all of the nineteenth century. In this connection the pioneer work of Ehrlich should not be overlooked for in his endeavors he founded a broader science, chemotherapy, which correlated the best that is in chemistry, biology, physiology, pharmacology and medicine. The names of living biochemists who have surpassed the work of the forefathers are a legion and through their efforts centers of biochemical research have been established and the progress of medicine has been enhanced by their efforts.

The newer biochemistry does not restrict itself to chemical constitution but includes the physicochemical aspects of the living process and through these interpretations based upon exact laws valuable aid in the diagnosis, prognosis, and treatment of disease is attained. It is however equally important

that these newer aspects should not so engross the attention of the investigators that the older problems are neglected or overlooked. Examinations of greater and greater accuracy have been demanded and it is pleasing to say that the application of an exact science has furnished the necessary measures.

Sometimes the spark of life burns brightly, at others it glows dimly and yet through these diversities chemical methods are able to throw light on our bodies which act as simple furnaces. If one gland or another is in a stage of hypo- or hyperfunction, if some special enzyme or hormone fails in its activities, these derangements manifest themselves in the form of disease. Thus an insight must be obtained by chemical means which aids the physician in diagnosing and treating this symptom. Thus glycosuria, albuminuria, uremia, icteric index, basal metabolism, cholesterolemia, uric acid, acid-base equilibrium all offer much useful information as to functional activities of certain organs. Through physiology and pharmacology the relation of vagotonic and sympathetic overbalance add to our knowledge of permeability, and electronic exchanges in the body. Likewise, the protective mechanism of one individual as compared with another is closely if not entirely related to the chemical differences invested in these living operatives. Furthermore I for one believe that certain maladies malignant in nature can be traced to chemical origin. The researches of biochemistry can without question throw light on morbid processes and with this light producing therapeutic products through whose administration relief from suffering, may be obtained.

No better illustration of this idea can be given than in the use of salvarsan in the treatment of syphilis, the application of gold in certain diseases of the skin, insulin in the treatment of diabetes, mercurials in the reduction of edema, novocain in surgical anesthesia. These are but a few of the coordinative studies of the nature of disease. Thus it is necessary for the biochemist to familiarize himself with disease, its symptoms and lesions, as well as for the physician to become acquainted with the basic principles of physics and chemistry as exact sciences. For example, metallic substances may form coordination compounds with trivalent arsenic and antimony compounds of the arsenobenzol type by virtue of their residual affinities. The therapeutic effect is enhanced through the presence of these nonionic colloidal metals. This is illustrated by silver-salvarsan in which the silver oxide is protected from aggregation by the emulsoid action of the parent arsenical. Here physical and colloidal chemistry finds a common meeting point with the production of a more highly active therapeutic substance.

Our rapid progress in the more exact method of measuring functional changes in the living organisms has been brought about by the honest endeavor of groups of individuals exchanging ideas and then applying them to diseases in question. Their efforts along this line have found application in the study of skin diseases or those which may produce cutaneous manifestations. Looking at it broadly disease is only a manifestation in which the forces of the invader have succeeded in overcoming the powers of resistance of the invaded and as a result, our studies have endeavored to measure the destructive manifestations visibly and obscurely left in the field after the attack.

In order to develop a therapeutic measure it is primarily necessary to know the abnormalities produced by the disease in question. In the second

place it is then necessary to devise compounds and their derivatives which will produce changes similar in nature to those which are missing. These abnormalities may manifest themselves by the absence of certain definite substances or by an excess of these same entities or still by functional changes which in some instances are more difficult to observe.

In our investigations of skin diseases the application of the well known principles readily placed themselves at our disposal.

For instance eczema a very common disease easily recognized by all showed very common physical manifestations namely in abnormal passage of fluid outward through the skin. This observation implied an abnormal regulatory phenomenon involving a semipermeable membrane. Regulation is the conservative method necessary for the free and independent life of the organism governed by necessary stimuli and reactions that result through this multiplicity of mechanisms which lead to phenomena designated as life. In all physical and physiologic processes the problems of volume, time, temperature and concentration enter into these exchanges of fluids through the cells and are governed by the complex principles of catabolism and anabolism associated with electronic exchanges. The cell, its wall and its contents involve the more detailed study of dynamic equilibrium, not static equilibrium, dysfunction or perversion of the normal regulatory processes leading to certain pathologic conditions of disease. Microscopic examination of cutaneous cells showed the presence of edema which is only a disturbance of the volume of the cell. This volume change of the cell could be interpreted only as a vital effort in the restoration of the electronic and osmotic balance.

With these ideas in mind our attention was directed to the more common skin diseases such as eczema, vitiligo, psoriasis, acne and the common dermatoses. In order to obtain the greatest number of values at any one interval it was necessary to fix the specimen so that it would not deteriorate and to obtain sufficient quantity so that a series of substances could be obtained simultaneously. It is our belief that for the first time our investigations have shown the importance of the chloride sugar ratio. In carrying out these examinations the following substances are determined as an aid in diagnosis: sugar, chloride, urea, uric acid, cholesterol and icterus index. This can be carried out in a breezy style and the report available within a few hours after the specimen has been obtained. No specimens are used unless immediately fixed inasmuch as investigation has shown that blood from the ice box is unsatisfactory for sugar and urea estimations. All the methods employed are checked by means of the addition of known amounts and recovery must be at least 95 per cent accurate. In other words adequate control on the methods and the solutions employed is essential. The normal values established by us are in terms of whole blood: sugar 90 to 95, chlorides 445 to 455, urea nitrogen 14.5 to 15.5, uric acid 2.7 to 3.2, icterus index 4 to 6, cholesterol 150. During the past year about 40,000 determinations have been made and through these studies it has been shown that a single set of determinations is almost useless. For this reason a weekly examination, sometimes more frequently if necessary, is made on every patient under treatment.

The various skin diseases previously enumerated and syphilis which occasionally shows cutaneous manifestations are accompanied by marked disturbances



in the functional equilibrium of the organs of the body. For instance the injection of arsenic preparations occasionally provokes marked disturbances of the involuntary nervous system. This disturbance can be measured by a study of the decrease of leucocytes in the peripheral blood and the accumulation in the splanchnic region. Furthermore psychic irritations (fear, joy, etc.), changes in temperature produce a change of the forms of activity of the skin: the skin becomes pale because of the involuntary contraction of the skin vessels. These changes cause the skin to become less permeable, water elimination through the skin is decreased as well as the electrical conductivity. This process is transient and reversible. When hypoglycemia exists the skin vessels involuntarily become dilated and the endothelial cells become highly permeable with an intensive perspiration.

With these physiologic principles in mind examination of individuals suffering from these diseases is carried out with the result that in eczema there are three well defined groups of cases illustrated by the following classification:

a. Metallic group showing a low chloride and a high sugar. Our investigations on this hypochloremia and a hyperglycemia have been confirmed by many clinicians. For instance arsenic intolerance, mercury intolerance readily produce this condition and the estimation of the metal easily confirms this finding. Values for sodium chloride in these conditions usually lie between 300 and 400 mg. per 100 cc. of blood and the accompanying hyperglycemia is 125 to 200 depending upon the severity of the condition. The values for urea and uric acid are seldom disturbed in the group.

b. Hyperglycemia group closely associated with diabetes without the presence of sugar in the urine.

c. Protein group with high uric acid moderately elevated or depressed urea with an increased icterus index.

These groups have been arrived at through the examination of more than 2500 patients and approximately 100,000 examinations. In psoriasis which has been under treatment, the biologic values for the most part fall in group A. Acne, furunculosis, and infections usually show a hyperglycemia and an increase in the uric acid values. Psoriasis is also accompanied by a hypercholesteremia. Details showing the classification of the patients by age, sex, color, etc., will be published in the clinical discussion of these values.

In syphilis, the patients showing an intolerance have a hypochloremia, a hyperglycemia, a decreased urea and a high icterus index. The values are a good indicator of alarming disturbances which may follow, and therefore treatment should not be continued. These findings emphasize the use of drugs that are highly active in small doses rather than depending on low toxicity and large doses. Low toxicity and low therapeutic value go hand in hand. Therefore it is necessary under these conditions to increase the dose to obtain clinical results which lead to late by effects. Furthermore, it shows that all of these conditions are closely associated with changes in the vagotonic and sympathetic balance which bring about alterations in the metabolism which can be measured by the substances mentioned above.

In conclusion it should be stated that chemistry and medicine are closely associated and by means of chemistry biologic changes may be accurately measured, thus affording valuable data in the diagnosis and prognosis of

pathologic conditions. Biochemical methods also afford an accurate means of following the progress of treatment as well as furnishing precautionary measures before instituting treatment either in skin diseases or syphilis.

For example experiments have demonstrated that the permeability of the vascular endothelium of the splenic region always shows clearly a reaction opposite to that of the peripheral organs and tissues of which the skin is only a part. The examination of the blood by means of biochemical methods is closely associated with clinical aspects. Pruritus is invariably associated with hyperglycemia edema with disturbances of the salt balance recurring ear-riena with hyperglycemia and overweight nephritis and hyperchloremia eczema with hyperglycemia hypocholesterolemia and hyperuricemia syphilitic disturbances with sugar chloride ieterus index and urea disturbances.

By means of systematic accurate routine examination of the blood of patients many preventable clinical symptoms may be avoided. There must be a determination to obtain the specimen absolutely fresh so that it is as near as possible to the clinical condition otherwise the results are useless. It is believed that dermatitis dermatitis exfoliativa and jaundice can always be detected long before their manifestation in syphilitic patients and thus avoidable. Metallic intolerance in association with metallic therapy can likewise be detected by a hyperglycemia and a hypocholesterolemia in other words that is a numerical decrease in the sugar chloride balance (1 to 5 normal). As a result of many thousand determinations a complete blood examination is essential as a routine procedure in dermatologic clinics as well as general clinical practice.

644 PACIFIC STREET

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## EFFECT OF COFFEE AND TEA ON GASTRIC SECRETION\*

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BY W. HORSLEY GANTT M.D., U. S. S. RUSSIA

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THE almost universal use of coffee and tea as beverages among civilized people is an important reason why we should thoroughly understand their action. In many European countries indeed they furnish the main water intake especially in Russia where the drinking of quantities of weak tea is a habit prevalent among all classes.

The purpose of our experiments was to find out the chemical action on the secretion of the stomach of coffee and tea when taken with food.

As is well known the secretion of the stomach to any substance which is eaten consists of two components the psychical or nervous secretion and the chemical secretion. This was shown first in 1852 by Bidder and Schmidt and later by the sham feeding experiments of Pavlov.<sup>1</sup> As the nervous secretion being a conditioned reflex varies with each individual according to whether he likes or dislikes tea or coffee and with many other factors we desired to eliminate this so as to get the pure chemical or unconditioned effect.

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<sup>1</sup>From the Physiological Laboratory of Prof. Pavlov at the Institut of Experimental Medicine, Leningrad.

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TABLE I  
COMPARISON OF TEA AND WATER ON GASTRIC SECRETION

150 GM. DISTILLED WATER PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA				150 GM. TEA PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA			
Order of experiment		1st	4th	Average	2d	10'	Average
Latent period		15 min	—	14	—	10'	14
First hour		30 cc	30 cc	28 cc	24 cc	24 cc	28 cc
Second hour		17	21	17	—	10	17
Third hour		14	21	17	—	10	16
Fourth hour		07	16	11	—	10	17
Total				70 cc			73 cc

TABLE II  
COMPARISON OF COFFE AND WATER ON GASTRIC SECRETION

150 GM. DISTILLED WATER PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA				150 GM. COFFEE PLUS 100 GM. BREAD INTRODUCED INTO GASTRIC FISTULA			
Order of experiment		1st	4th	Average	2d	10'	Average
Latent period		24	32	28 min	17	20	22
First hour		11 cc	10 cc	15 cc	20 cc	16 cc	17 cc
Second hour		06	05	05	09	07	06
Third hour		05	—	05	16	15	10
Total				07			107

We first tried to introduce the mixture of bread and tea into the stomach through a stomach tube. Here we met with several difficulties: the food passed slowly along the tube and often clogged; it was difficult to prevent the animal from seeing the food and tasting it when the tube tip was pulled out over the tongue; the introduction of the tube evoked the passive defensive reaction in the dog, which might have a disturbing influence on the gastric secretion.

Our dog had already a stomach pouch according to Pavlov's method, so that the secretion could be collected and measured accurately. In order to overcome the above mentioned difficulties we made a gastric fistula in the large stomach and began our experiments about three weeks after operation when the secretion had returned to normal.

Our dog was about three years old and had been used in the laboratory for more than a year previously, having shown a constant and regular gastric secretion.

The experiments were done as follows: no food was given the morning of the experiment, the last feeding being on the previous evening at 6 P.M. The stomach was first washed clean with ordinary water at room temperature; if there was any secretion we waited until it disappeared or until the contents of the stomach became alkaline. The dog was put on its back and the bread and tea mixture was introduced through the gastric fistula. Every precaution was taken to keep the animal from seeing and smelling the food in order to prevent any psychical secretion.

The dog was returned to the stand; the latent period noted by testing with litmus; the reaction in the stomach pouch and the gastric juice therefrom collected and measured every fifteen minutes.

In order to eliminate any daily variation in the secretion control experiments were done on the alternate days using a mixture of bread and a volume of water equal to that of the tea. Both the tea and coffee were made very strong by boiling for five minutes 3 to 5 gm. of tea or 5 gm. ground coffee with 150 cc. of water. Three experiments were done in a week on alternate days.

The results of our experiments are shown in Tables I and II. (These tables are not to be compared with each other as the experiments with coffee and tea were done at an interval of several months.)

#### DISCUSSION

In the human Miller, Fergheim, Rehfuess and Hawn<sup>1</sup> compared the action of 1000 cc. of coffee or tea with water. This amount of coffee and tea plus the test meal of 100 cc. they found produced nervous symptoms such as palpitation, flushing, shivering, etc. and delayed the secretion but finally the same acidity was reached as from the test meal alone (cited by Lablanc<sup>2</sup>).

Kestner and Warburg<sup>3</sup> found in a dog with a duodenal fistula that coffee produces a slight gastric flow and tea very little.

Kestner and Knippinger<sup>4</sup> state that the action is due only to those substances entering into solution: i.e. 25 to 30 per cent of the weight of roasted coffee. In 25 gm. of a watery extract made from 100 gm. of ground coffee there are,

besides other things 1.7 gm of nitrogenous compounds about 1.5 gm of which is caffeine, also 5.2 gm of oil and 4.1 gm of mineral substances

Thirty to 40 per cent of the weight of tea enters into solution and 2 per cent of the resulting solution is caffeine. Thus in a cup of coffee made from 7.5 gm of coffee there is 0.1 gm caffeine but in a cup of tea prepared from 2 gm of tea leaves, only 0.02 gm caffeine. These authors remark that nothing of nutritive value is present neither albumin nor vitamins and that their effect comes from the action of the caffeine on the nervous system and on the gastric secretion. Bickel and Eweck however state that the influence on the stomach secretion is not from the caffeine but from the products arising during the process of roasting.

The use of coffee and tea as palatable beverages taken with food should not be overlooked. In this capacity they mix and digest in two ways. First, by the stimulation of the "psychical" or nervous juice and second, by adding to the water intake. That the nervous secretion may be considerable has been well established by the sham feeding experiments carried out in the laboratory of Prof Pavlov. The gastric juice obtained by five minutes of fictitious feeding in a dog with an esophageal fistula so that none of the food enters the stomach but is only swallowed and dropped out from the fistula may last for three hours and almost equal that produced by bringing the food directly into the stomach through a fistula i.e. the chemical secretion.

As regards the water intake on the flow of gastric juice Prof Pavlov says "If tea and coffee through their water content help to supply a water deficiency in the body they may become extremely important factors in digestion. In my experiments with sham feeding it was shown that if the animal was deprived of fluid the gastric secretion gradually sunk and after several days became zero. As soon as water was given, the secretion began. If the water intake is too low all of the secretions will be greatly decreased" (Personal communication for this paper). Of course from this point of view, coffee and tea have no specific value, and are of use only in so far as they insure a sufficient amount of water for digestion and they could be substituted by any other aqueous beverage provided it were taken in the same quantity.

#### SUMMARY

The chemical action of a mixture of tea and bread on the stomach secretion was found to be practically the same as a mixture of bread with an equal amount of water. A mixture of coffee and bread produced a slightly greater amount of gastric juice during the first two hours. The latent period (the beginning of the secretion) was not affected in either case. The increase in juice was very little, 0.3 c.c. for the first hour in the stomach pouch, or about 3.0 c.c. for the whole stomach. The nervous element was eliminated by introducing the mixture through a gastric fistula and the collection of the juice was from a miniature Pavlov's stomach.

We may conclude that the effect of even very strong coffee and tea on the stomach secretion depends almost entirely upon the individual i.e. upon the nervous secretion, and upon the water content, there being little or no chemical influence due to the tea and coffee per se.

## REFERENCES

- 1 Pavlov, I. I. *Work of the Digestive Glands* London 1910 p. 7
- 2 Miller, Berghum, Rehfuess and Hawk. *Am J Physiol* 52: 28, 1920
- 3 Babkin, B. I. *Die aussere Sekretion der Verdauungsdrusen*, p. 392 Berlin 1908
- 4 Kestner und Warburg. *Klin Wchnschr* 2: 1791 1923
- 5 O. Kestner und H. W. Knipping. *Die Ernährung des Menschen*, Berlin
- 6 Bickel und von Fweyk. *Ztsch f l ges ext Med* 54: 7, 1927
- 7 Pavlov, I. I. *Work of the Digestive Glands* London 1910 p. 100

## CALCIUM DEPOSITION IN TISSUES OF DOGS AND MICE BY THE AID OF PARATHORMONE\*

BY ALTON LEAFNER M.S. CHICAGO, ILL.

### I. INTRODUCTION

THE development of a potent extract of the parathyroid glands ended a long search for a hormonal substance of this gland. This substance has given rise to many studies on calcium metabolism, the parathyroid glands and to therapeutic studies.

It has been forty eight years since Sandstrom (in 1860) first discovered the parathyroids, but for many years then great significance lay in the role they played in preventing tetany by their presence in whole or in part in the body. The work with these glands was related to describing the picture of parathyroid tetany. In later years, however, chemical studies of the glands and of the blood serum were undertaken which led to the accumulation of the data now known and accepted more or less. At various times the explanation of the function of the parathyroids was attributed to a detoxicating action against guanidine and methyl guanidine and then again by others to an influence in the regulation of the calcium metabolism, and by others still to a regulation of the acid base equilibrium with disturbance thereof in parathyroidectomies causing the symptoms of tetany.

While there is no fully adequate explanation of the modus operandi of the parathyroid glands, the influence of these glands on the concentration of calcium in the blood is given full recognition. Successful preparation of the parathyroid glands has been developed by Hanson, Beermann, Collip and others. Injection of these preparations results in the development of a hypercalcemia with a train of symptoms namely, anorexia, emesis, increasing somnolence and phenomena of circulatory weakness. This has been described by many workers but especially by Collip whose preparation and standardization of the product known as parathormone is probably the most significant work of increasing our knowledge of these glands.

The most striking results of parathormone injection have been obtained on dogs. Subcutaneous or intramuscular injections depending on the dose may raise the blood serum calcium well over 100 per cent. Positive, though not so

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stating results have been obtained in calves, cats and rabbits. These latter though showing a hypercalcemia are relatively resistant, for they do not develop the serious symptomatology attendant on the hypercalcemia in the dog.

Several questions are raised by these observations. What is the cause of the hypercalcemia, and what are the possible consequences of the increased calcium content? There is as yet no adequate answer to these questions.

Greenwald and Gross have hypothesized the existence of a calcium dissolving substance which is either parathormone itself or a substance developed as a result of its action. This substance is necessary to keep in solution the excess of  $\text{Ca}(\text{PO}_4)_2$  which Holt, LaMer, and Chown have shown to be present in the blood.

Stewart and Percival proceeded to answer the questions raised by the calcium raising power of parathormone in a series of interesting experiments. They showed that parathormone raised the calcium in the blood serum even when the factor of any influence on absorption or excretion was eliminated by evisceration. They also reached the conclusion that the liver, spleen, pancreas, pituitary, thyroid, and central nervous system have no special function in connection with the effect of the parathyroids on the blood serum calcium content. They came to a conclusion which is in agreement with that by Greenwald, namely, that the calcium drawn into the blood comes from the soft tissues or from the bone, or both.

There is that other question to be answered, as to the consequences of the hypercalcemia. We have mentioned the clinical picture which develops. Are there any pathologic consequences?

M. B. Schmidt in his discussion of pathologic calcifications divides the same into two main groups:

1. Calcifications occurring where there have been local tissue disturbances without any change in the calcium metabolism, and
2. Calcification occurring in healthy tissues as a result of changes in the calcium metabolism.

Important factors are:

- a. Oversaturation of the blood and tissue fluids with calcium.
- b. Inability on the part of the excretory organs to excrete calcium satisfactorily.
- c. Changes in the solubility relations in the blood for calcium.

In the different studies on the chemistry of the process of calcification various conditions were considered as important. These were, (1) the formation of calcium soaps, (2) the combination in some way of calcium with phosphoric acid, (3) the physical adsorption of calcium salts, and (4) calcification as a simple precipitation. The last two gain most support now.

"Hofmeister advances the hypothesis (Wells' *Chemical Pathology*) that when the cartilage or other matrix becomes saturated with calcium salts, any decrease in  $\text{CO}_2$  content of the solution will lead to a precipitation of calcium salts, thus restoring to the cartilage its power of absorbing more calcium salts whenever the fluid comes to it with a higher degree of saturation with calcium salts and  $\text{CO}_2$ . In support of Hofmeister's idea is the fact that a similar fluctuating effect, produced by alternately varying the reactions by feeding acids and bases, was found by Rabl to lead to metastatic calcification."

Rabl fed, alternating every two days to 1 group of mice, first a diet yielding an acid ash, then a diet yielding an alkaline ash. At the same time he increased the calcium content of the food. His view was that the blood overloads itself with calcium when there is a reaction of acidity; then there is a precipitating out of the calcium with a developing alkaliscence. Dreyfuss subsequently repeated the experiment of Rabl with some slight modifications in that he had three additional groups, namely acid ash diet alone and alkaline ash alone, these having increased calcium and a group maintained on the usual diets. Metastatic calcifications were found in all the groups, though to a lesser degree in the latter groups. Even in these however some calcification was found in the kidney and lung (in the lung the calcium being mainly limited to the bronchial cartilages). Dreyfuss makes mention in the interpretation of the results, and the transference of inferences to other animal types and he states that calcifications in mice are not unusual findings under any circumstances.

Rabl's work lays stress on and supports the fact of the importance of alternating reaction. The question arises as to the possibility of calcifications in a healthy organism through simple overloading with calcium. Katase was able by simple injection (subcutaneous, intravenous and intraperitoneal) without any previous tissue injury to produce calcifications in almost all tissues and organs. He used guinea pigs and rabbits.

Katase noted in addition to the calcification of the elastic membranes of the alveolar septae of the lungs some interesting findings in the bronchi. He found the bronchial mucosa more or less desquamated. The epithelial cells showed in their cytoplasm numerous inclusions of calcium granules presenting a picture quite similar to that presented by mucous production. This picture he found to be identical with that of the colon mucosa. He found calcium granules in the lumen. He concludes therefore, that the bronchial mucosa can serve a calcium excretory role and continues to say that if the calcium granules came from calcified lung masses there should appear in the sputum incrustated tissue elements and the lungs should also show evidence of breaking down. The significance of bronchi as excretory organs for calcium was first established by these experiments.

In the intestine the calcification was in the connective tissue, muscularis, muscularis mucosae and in the membranae propriae of the glands.

In the liver the calcified liver cells were in groups of different sizes and were found in almost any part of the veni in different places. There is as it were a network of calcified liver columns.

Of the tissue elements of the kidney the epithelium of the convoluted tubules suffered most and then the interstitial tissue as the membranae propriae. The explanation offered for these results is that so long as the calcium is excreted in a normal fashion there is no calcification but that when the excretion is interfered with or the calcium content of the body fluids exceeds the excretory capacities of the kidney and intestines then there results a precipitation of calcium in the different tissues and organs.

Thus we see that calcification can occur after injection of calcium salts. In parathormone we have an agent that can mobilize calcium. Hineper reported the findings of metastatic calcification in dogs by its use.



We have repeated the experiment, using 100 to 150 units parathormone, Collip, with results that are to some extent similar to Harper's. We however, have some very interesting additional findings to report. It was interesting to note the tendency of the calcium to be deposited at different sites of election in the different organs.

#### II. EFFECT OF PARATHORMONE ON DOGS

Since our chief aim was to study the effect of parathormone on mice we used only two dogs as controls. The dogs developed a hypercalcemia of 16.77 and 19.57 mg. per 100 cc. respectively. The latter dog showed very pronounced calcium deposition, while the dog with the smaller rise showed a lesser degree of calcium deposition.

Macroscopically little was to be seen except that the blood was clotted and that the stomach showed evidence of a spastic state by the marked rugae

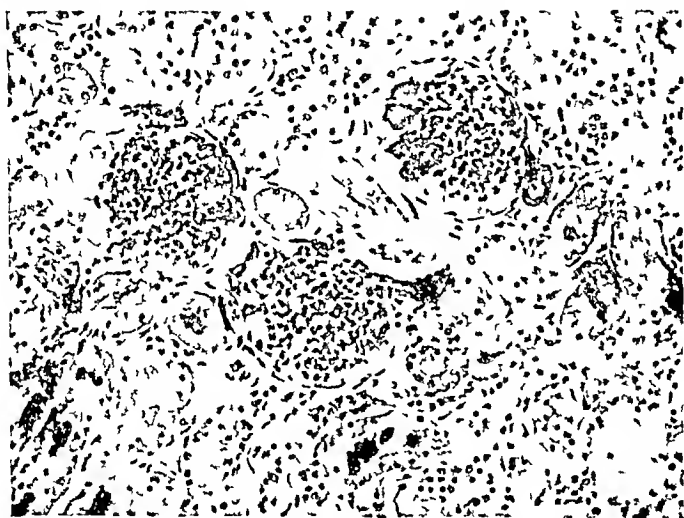


Fig. 1.—Kidney from a dog. (Kossa stain counterstained with Hemalum Fast.) Calcium shows up black and is seen in Bowman's space in the lumen of some of the tubules and in some of the epithelial cells. A calcified artery is seen and some calcium is present on the basement membrane of some of the tubules.

formation. The stomach and intestinal mucosae appeared hyperemic but there was no evidence of extravasation of blood.

*The Kidney* (Fig. 1) showed on microscopic examination the following:

For the most part Bowman's capsule appeared as an unaltered, distinct single cell layered epithelium. The cells of the glomerules were of good staining quality. A few of Bowman's capsules showed calcium deposits. Much calcium, however, was found in Bowman's space, and between the loops of the glomerular tufts. There was a granular debris in some of the Bowman's spaces.

The tubular epithelium where there was no deposition of calcium showed no alteration from the normal staining qualities. Where, however, the tubular epithelium showed calcification, there it presented an appearance of necrosis, the change in the cells being directly proportional to the amount of calcium

The calcium deposition in the epithelium proper varied from a few granules to a solid mass. Most of the epithelium however was unaltered. It was in the lumen of the tubuli where the calcium was most to be seen. The granular debris seen in some of the Bowman's spaces was present in some of the tubules. There was some calcium on the basement membranes of the tubules, and also in the walls of some of the arteries and capillaries.

*The Liver* (Fig. 2) Cells about the portal canals as a rule were unaltered, those centrally however showed a slight degree of atrophy, probably due to the passive congestion which was present. The calcium was deposited in the form of granules which were present in greater or lesser amount in the parenchyma cells and to a lesser extent in the Kupffer cells. The localization of the



Fig. 2—Liver from a dog (Stained as Fig. 1) Calcium granules in liver cell and in a Kupffer cell

calcium seemed to correspond to the areas where congestion was more marked, namely centrally. In places the calcium was in the capillary walls adjacent to the liver cells.

*The Lungs* (Fig. 3) in addition to showing calcium deposits in the elastic fibers of the alveolar septa where it has already been described by others, showed a very marked deposition of calcium in the bronchial walls on the elastic fibers and in the tunica propria of the bronchi. The bronchial epithelium showed no calcium in it and it was desquamated. The bronchial cartilage was free from calcium. Small hemorrhages were noted.

*The Heart* showed considerable calcium deposition between the muscle fibers. The individual cardiac muscle fibers were seldom affected. The intima of the coronary vessels showed calcium deposition.

*The Spleen* was unaltered except for the deposition of calcium in the intima of the larger branches of the splenic artery immediately below the endothelial lining.

Distilled water was used. The calcium free diet ( $\text{Ca}^-$ ) contained 0.008 per cent of calcium. The control food ( $\text{Ca}^+$ ) contained 0.546 per cent of calcium and the rich calcium food contained double the amount of calcium ( $\text{Ca}^{++}$ ) in the control food. The lactose salt mixture was based on the composition of the salts of milk, and the ash of the entire mixture varies only slightly from neutral reaction.

A few of the mice died before the end of five weeks, but the majority were killed at the end of the five weeks and pieces of the following tissues were taken for section: heart, lung, liver, kidney, spleen, adrenal, stomach, brain, and femur. The pieces were fixed in alcohol and stained by Kossa's method. As counterstain, hemalum or hemalum eosin were used.

Microscopic examination of the organs revealed negative results except in the bronchial cartilages (Fig. 4). These were calcified in the majority of

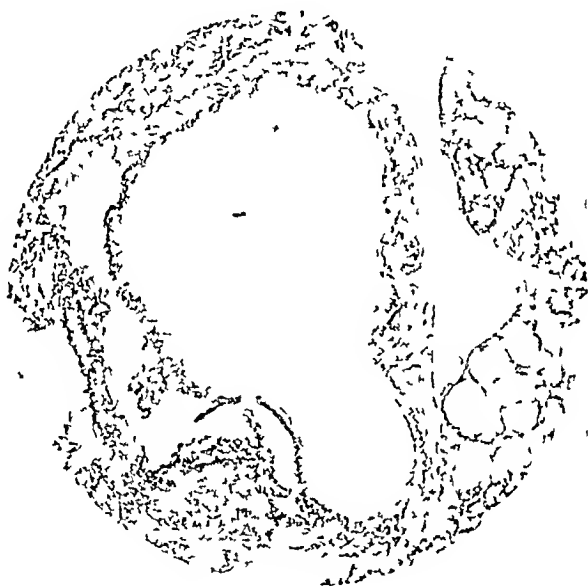


Fig. 4.—Lung from a mouse (Stained as Fig. 1). Calcium deposition only in bronchial cartilage. None elsewhere.

cases, varying in degree from slight to very much. It is to be noted that normally the bronchial cartilages of mice show a degree of calcification which though usually not very intensive, is nevertheless positive. In our material it seemed that the intensity of bronchial cartilage calcification was more marked in the injected than the noninjected mice. It seems thus that with parathormone and calcium, what occurs is an intensification of what is for mice an apparently physiologic process. These findings, however, cannot be stressed as significant from the standpoint of the calcium raising power of parathormone in mice, or as bearing any evidence for calcification in mice resulting from parathormone administration.

Several mice were also injected with 10 units parathormone. When one realizes that these mice averaged 20 gm. in weight one readily recognizes what an incredibly large dose these mice received. Yet after forty-eight hours of

observation these mice manifested no signs whatsoever of any ill effects. His histologic examination supported the observations during life. No other calcifications than those of the bronchial cartilages were seen.

While we were making the histologic studies of the effect of parathormone on white mice Sussmann reported some chemical studies on white mice with parathormone. He determined the normal blood serum calcium content of the white mouse to be about 18 to 20 mg. per cent. He then injected parathormone Collip in doses of 10 to 20 units and was unable to notice any effects on them. He points out that a dose of 10 units for a mouse is the equivalent of a dose of 20,000 units for an adult of 64 kg. and he remarks that such dosage speaks for itself. Thus we see in Sussmann's studies chemical findings which support our histologic findings for he was unable to detect any rise in the blood serum calcium content or if there was a rise it was very insignificant.

Attention is called to the fact that the resistance of white mice to injections of such character is not new for Voegtlin and Dyer have shown that white mice and also white rats are capable of enduring doses of epinephrin and pituitary extract 100 to 1000 times the usual dosage for other animals. To account for this peculiarity in the present state of our knowledge is an impossibility and we must for the present pass over it vaguely by stating that the white mouse possesses an especial resistance to such hormonal substances.

Katze makes the observation that the intensity of calcification is inversely proportional to the normal calcium content. The calcium tolerance of each organ must vary. Quite possibly the organs with high calcium content already under physiologic conditions possess an especially high calcium metabolism and are therefore able to take care of a large influx of calcium without any resulting precipitations. The organs on the other hand with low calcium content may not be able to cope with a high calcium influx therefore calcification occurs.

It may be that the findings of a high calcium content for the mouse speak for an active calcium metabolism and an ability on the part of the mouse to handle a calcium increase without precipitation or evidence of rise in content. Further study is necessary.

#### SUMMARY

A histologic study of the experimental calcification in dogs produced by injections of parathormone gave the following results:

In the kidneys the calcium is found in the Bowman's space of the glomeruli in the lumina of the tubuli and to a lesser degree in the tubular epithelium. Some also is present in the wall of the smaller arteries. In the liver calcium is deposited in the form of granules in the liver cells of the central portions of the acini and also in the Kupffer cells. In the lungs calcium is observed in the wall of the bronchi below the epithelium and in the alveolar septa. In the heart the calcification involves chiefly the interstitial tissue between the muscle fibers while much less is found inside the muscle fibers. In the intestine calcium can be detected in the connective tissue about the basal portions of the glands. Some of the desmorphic cells of the stomach are seen filled with calcium.

These microscopic findings are explained on the basis of either a fluctuation of the reaction with alteration in the solubility for calcium, or as the result of a simple overloading with calcium.

Mice injected with large amounts of parathormone show only an increase of the physiologic calcification of their bronchial cartilage. The otherwise negative histologic findings are in accord with the great resistance of these animals against parathyroid extracts.

#### REFERENCES

- 1 Boothby, W. M. The Parathyroid Glands, *Endocrinology* 5: 403, 1921.
- 2 Collip, I. B. The Parathyroid Glands, *Medicine* 5: 1, 1926.
- 3 Schulten, H. Das Hormon der Nebenschilddrüsen. *Klin. Wochenschr.* 4: 2457, 1925.
- 4 Schmidt, M. B. Die Verkalkung, Kiehl und Marchand, *Handbuch der Allgemeinen Pathologie* III 2, p. 215, 1921.
- 5 Aschoff, L. Veränderungen des Kalkgehaltes, *Pathologische Anatomie* 1: 425, 1925.
- 6 Stewart, C. P., and Percival, G. H. Action of Parathyroid Hormone on the Calcium Content of the Serum and on the Absorption and Excretion of Calcium, *Biochem. J.* 21: 301, 1927.
- 7 Rühl, C. R. H. Zum Problem der Verkalkung. *Virchows Arch.* 245: 542, 1923.
- 8 Dreyfuss, W. Über den Kalkstoffwechsel im Tierversuche. *Beitr. z. path. Anat.* 76: 254, 1927.
- 9 Katase, A. Experimentelle Verkalkung im Gesunden Tiere. *Beitr. z. Path. Anatomie* 57: 516, 1914.
- 10 Greenwald, I., and Gross, J. Effect of long continued Administration of Parathyroid Extract Upon the Excretion of Phosphorus and Calcium. *J. Biol. Chem.* 68: 325, 1926.
- 11 Greenwald, I., and Gross, J. Effect of Administration of Potent Parathyroid Extract Upon Excretion of Nitrogen, Phosphorus, Calcium, and Magnesium. Solubility of Calcium Phosphate in Serum and the Pathogenesis of Tetany. *J. Biol. Chem.* 66: 217, 1925.
- 12 Heinricher, G. Epithelkörperchenhormon und Kalkstoffwechsel, *Verhandl. d. deutsch. path. Gesellsch.* 22: 210, 1926.
- 13 Matthews, S. A., and Austin, W. C. Effect of the Blood Calcium Level and Tolerance to Magnesium, *Am. J. Physiol.* 79: 708, 1926.
- 14 Hueper, W. Metastatic Calcification in Dog After Injection of Parathyroid Extract. *Arch. Path. & Lab. Med.* 3: 14, 1927.
- 15 Sussmann, H. Experimentelle Studien mit Parathormone—Collip in Weissen Mäusen, *Ztschr. f. d. ges. exper. Med.* 56: 817, 1927.
- 16 Osborne, T. B., and Mendel, L. B. The Inorganic Elements in Nutrition, *J. Biol. Chem.* 34: 131, 1916.
- 17 Wells, H. G. *Chemical Pathology*, 1925, ed. 5, 486, W. B. Saunders, Philadelphia.
- 18 Hedinger, E. Demonstration einer Leberverkalkung, *Zentralbl. f. allg. Pathol.* 20: 1031, 1909.
- 19 Voegtlin, C., and Dyer, H. A. Natural Resistance of Albino Rats and Mice to Histamine, Pituitary, and Certain Other Poisons, *J. Pharmacol. & Exper. Therap.* 24: 101, 1925.

## EPINEPHRIN ADDICTION IN BRONCHIAL ASTHMA\*

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IT IS a common custom to permit or even urge the chronic asthmatic to treat himself with epinephrin in order to relieve the severe paroxysms. If the patient is unable to give the injections to himself some member of his household is usually trained to perform this service. It must be admitted that this procedure has spared the asthmatic much suffering and not a little expense. Certain objections however are inherent in the method. These I shall attempt to point out with the aid of the following brief case histories.

**CASE 1**—Miss M., white female. Aged twenty three years. Scotch ancestry.

**Past History**—Patient dates the onset of asthmatic symptoms from a fall off a horse several years ago. This did not result in the fracture of any bones but she was confined to bed for a few months. It was said that she had injured her neck. More detailed questioning revealed that it was really two to four months after the injury that the first attack of dyspnea occurred. The onset did not appear to be associated with a respiratory infection or with any communicable disease. The subsequent attacks could not be correlated with season climate or other extrinsic factors.

**Physical Examination**—The findings were typical of those in bronchial asthma. The patient was also poorly developed and nourished.

**Sensitivity**—Allergic skin tests revealed a moderate degree of sensitization to significant pollens and to feathers.

**Treatment**—She had been put on epinephrin therapy by her local physician a year or more previously. The injections were given by her sister who was therefore obliged to be a constant companion of the patient. Specific pollen therapy was tried and at first benefit was obtained but later it seemed to aggravate her condition.

**Subsequent History**—Almost complete relief for several weeks was obtained when an aunt and uncle visited the patient. A few days before the departure of her relatives her asthmatic symptoms returned. One very striking incident which occurred during this period seems worth recording. The patient was acting as "sight seeing" guide to her relatives on an automobile trip. The uncle was driving the car and the patient was busily occupied consulting maps directing him which road to take and pointing out and discussing points of interest. After a few hours of driving a site was selected for a picnic supper. Almost immediately after the car stopped the patient had a severe asthmatic attack. She had been entirely free during the ride and as this attack started before she got out of the car exertion did not seem to be the precipitating factor. Epinephrin was required to relieve her. No clue could be obtained to any environmental factor that might have caused the attack.

A day or two after her relatives had departed she was having nearly constant asthma. She had received no specific pollen therapy for several weeks. During the next two weeks I was called to her home several nights in succession and always at about the same hour. Her sister had been giving her large amounts of epinephrin but the patient obtained little relief. A smaller dose out of my bottle of epinephrin would often relieve her though occasionally it was necessary to resort to the use of opiates.

The amount of epinephrin had to be increased daily and its effectiveness seemed to decrease about as rapidly. Soon she was taking an ounce every three or four days in spite of which she would enjoy but short periods of freedom from symptoms. The large amount of this drug though failing to relieve the dyspnea seemed to bring about a lowered function of her several systems especially the gastrointestinal tract.

She was placed in a hospital and an attempt was made to reduce the total amount of epinephrin used by giving smaller doses of the drug, repeating more frequently if necessary. It was noted that the injections were repeated at nearly identical hours each day. This fact and the observation that at times smaller doses of the drug from my bottle would be effective, suggested the use of psychic therapy. This was started and hypodermics of sterile salt solution were substituted for the active drug.

The first night that epinephrin was withheld she was given a hypodermic of morphine sulphate 16 mg., and atropine sulphate 0.44 mg. These things had been tried before but had not effected a reduction in the amount of epinephrin. For the next few days it was necessary to repeat the hypodermics of salt solution at about the same intervals she had previously demanded the active drug. If the salt solution did not give satisfactory relief small doses of epinephrin were employed.

She was discharged within two weeks free from asthma and she had used no epinephrin for several days previously and required but few hypodermics of salt solution during my twenty-four hour period. The improvement in this case can definitely be dated from the time salt solution was substituted for epinephrin. Her sister was instructed how to continue the psychic therapy after the patient returned home. Her general condition as well as her asthma, continued to improve and she was symptomless for several months without any form of therapy.

**CASE 2**—S. G. white male Irish ancestry. Patient gave his age to one physician as fifty-two to another fifty-four years. The actual age was sixty-two, and his general appearance was more consistent with the actual age.

**Past History**—First attack of paroxysmal dyspnea occurred three years previously following a cold. No specific etiology was indicated by the history. The attacks seemed unrelated to season or climate in fact they were nearly constant. He was forced to give up his business because of the severe asthma. Excitation often precipitated an attack though he was willing to admit that he seldom had an attack when he was deeply interested in something, even though it did entail a certain amount of exertion.

**Physical Examination**—The findings were characteristic of those in bronchial asthma but emphysema was more marked than would be expected in one who had had asthma for so short a time. Circulatory findings at several examinations were not suggestive of any disturbance of the circulatory system. He was poorly developed and nourished.

**Sensitivity**—Routine food, pollen and other allergens were tested by the scratch and intracutaneous methods and no sensitizations were demonstrated. He stated that at one time he had reacted to horse dandruff.

**Treatment**—This patient had been using epinephrin for about two years and required frequent injections at night.

**Subsequent History**—The patient's wife had been visiting in the East for several weeks. For about two weeks before the date she was expected to return the patient had been having very severe asthma. He had been in bed at home and required practically hourly doses of epinephrin day and night. Often it was necessary to repeat a dose of 8 or 10 minims two or three times a few minutes apart. Two oculic solutions syringes were kept busy administering to this patient.

He was placed in a hospital in July, 1928. A short time after his admission he had a severe paroxysm. His condition was markedly aggravated because he did not receive the instantaneous service that he was accustomed to at home. This attack was controlled with difficulty. The next day he accused his physician and nurse of neglect and the hospital for having such poor service. For several days small doses of epinephrin were used and repeated in fifteen minutes if relief was not obtained. Frequently it was necessary to give a total of 23 to 25 minims in a period of thirty minutes before he would admit that he was relieved.

A study of his chart disclosed that certain hours each day appeared to be critical periods. These centered about the time his visitors left in the afternoon and also between 11:00 P.M. and 1:00 A.M. The reason for the latter period will be suggested later. For several days he averaged from 98 to 147 minims of epinephrin in twenty-four hours. Ten

minims was the maximum dose. Symptoms were getting definitely more severe, and he was advised that a change in the method of giving the drug might yield better results. He was informed that he would be given larger doses but at less frequent intervals.

At midnight that night he had a severe attack and was given 10 minims of sterile salt solution. Just before the injection he asked his nurse how much she was going to give him. He was told the amount but not the contents of the injection. He protested against such a large dose but the nurse insisted that it be given as ordered. Very shortly thereafter he experienced the signs and symptoms of overdosage of epinephrin. He stated that it felt as though there was a powerful explosive force inside attempting to blow off the top of his head. He became extremely nervous and said that his heart was beating at an excessive rate. He was so profoundly affected by this excessive dose of salt solution that the nurse feared the dyspnea might become worse. She therefore suggested that he take another small injection; this was to have been epinephrin. He refused this and stated that he would rather wheeze than take another injection at this time. In a few minutes he was asleep and practically relieved of dyspnea.

At 3:00 A.M. he asked for another injection and was given 5 minims of salt solution with relief of symptoms. At 6:30 A.M. he asked for another hypodermic and was given 8 minims of epinephrin without first trying to obtain relief with salt solution. When I visited him that morning he completely misled me by his description of the toxic reaction. Later when I consulted his chart I learned that he had not received active drug. In this first twenty-four hour period after salt solution was started he received but 44 minims of epinephrin. This figure seems quite significant in comparison with the amounts previously used. The next night he was given an injection of pantopon 22 mg. This drug had been used before but would not act as a substitute for epinephrin. For the next three twenty-four hour periods he required 24, 31 and 16 minims respectively. His private night nurse was then dismissed following which the amount of epinephrin used increased a little.

He was discharged a week later as he was having practically no asthma and his general condition was much improved. His wife who returned from the East the second day he was in the hospital helped to continue the psychic therapy after his return home. She reported several weeks later that the salt solution was still quite effective. Recently an acquaintance reported that the patient was so much improved that he looked and acted like an entirely different person.

The psychic factors in this case are too involved to justify a detailed discussion of them. A few additional points may be of interest. He is married to a woman over thirty years his junior—a possible reason for mistaking his age. At the time of his marriage about eight years ago he was a successful business man. He was then in a position to command and undoubtedly did so immediate response from his subordinates. Now he commands similar attention whenever he has an attack of asthma. He and his two unmarried sisters form an unusual mutual admiration triad. The sisters openly express their feeling that the patient's wife has stolen the love of their brother and that God never made a finer man than he. On the other hand the patient intimates that his wife is not sufficiently appreciative of the virtues of his sisters.

On one occasion when he 'staged' I use the word advisedly an attack just before the four of them were to sit down to supper the sisters became hysterical as a result of his violent wheezing. They refused to eat and returned to their home. His wife gave him several injections of epinephrin and when he was relieved she proceeded to eat supper. The next day his sisters emphasized to him how tender-hearted they were and how his suffering had completely abolished their appetite.

In repeating this to his wife he intimated that anyone who could eat supper when he had been so ill was very heartless. On the few occasions that his wife attempted to visit friends in the afternoon he would invariably have an attack about the time she was ready to leave. Then he would insist that she could not leave him in his present serious condition. Epinephrin would be resorted to and then he might be free for the rest of the day.

The critical period from 11:00 P.M. to 1:00 A.M. previously mentioned was investigated. While in the hospital his nurse observed that he would awaken during this period without having dyspnea but shortly after he would begin to wheeze and would request a hypodermic



This habit of awakening at this period did not seem to be dependent upon the type of weather. Ultimately it was learned that at home he would, at about the same hours, call his wife from an adjoining room and request an injection. If she started to go back to bed after he was more comfortable he would insist that she should read to him. Frequently she would sit beside his bed and read to him from midnight until two or three o'clock in the morning. These findings seem to make clear that a psychic mechanism was, in a large measure, responsible for his situation. Possibly a similar mechanism was operating in Case 1 when her attacks began just before the departure of her relatives.

**CASE 3**—Miss C, white female, aged twenty-five years. Ancestry is a mixture of French, English, and Irish. Occupation, nurse. She was admitted to the Los Angeles General Hospital a few days before I saw her. On admission she was considered critically ill and she was given a special nurse. The patient stated that she had been taking large amounts of epinephrin for the past two weeks but that it gave her practically no relief. Because of this the drug had not been administered previous to the time I saw her. She had, however, received an enormous amount of supportive and stimulative treatment.

At my first visit she was connected up to rather an elaborate and impressive arrangement of apparatus used to give her oxygen. A tube from this apparatus was kept in her nose by adhesive tape and oxygen was frequently supplied. Not wishing to have the patient exert herself even to the extent of answering routine questions I asked her sister to come out to the ward desk and supply the desired information. This produced an unexpected outburst of temper from the patient, and she made it quite plain that she knew her own history and preferred to give it. This demonstration immediately precipitated a severe paroxysm of wheezing. She became cyanotic and the oxygen was turned on.

Her special nurse was instructed to begin, as soon as possible, the use of psychic therapy combined with hypodermics of sterile salt solution, and to use no opiates unless absolutely necessary. At 8:15 P.M. that evening, August 10, she was given 2 minims of epinephrin and 6 minims of salt solution. This gave considerable relief. At 11:00 P.M. she needed another injection, and made a special request that something be used so that the hypodermic would not be so painful. She was given codeine sulphate, 4 mg. and 2 minims of epinephrin. At 2:30 and 3:00 A.M. she received 10 minims of epinephrin. Unfortunately, salt solution was not tried first, but she was having severe dyspnea and so the nurse thought the patient needed active drug.

The day nurse's notes in the chart for August 11 read "had a very good day." There was marked improvement from her condition in the previous twenty-four hour period. August 12 at 3:30 A.M. she was given 10 minims of epinephrin. For this twenty-four hour period the notes read "a very good day." At 11:40 A.M. she was given another injection of 10 minims of epinephrin. She was disconnected from the oxygen apparatus within a few hours after psychic therapy was begun, and was up in a wheelchair August 13, less than seventy-two hours after this method of treatment was started.

Her condition improved so markedly in this period that it seems improbable that it could be due to the total of 22 minims of epinephrin she received in twenty-four hours. It should be remembered that she had been taking huge doses of the drug for a week or two previously without evident relief. If this small amount of the active drug was the important factor in her improvement then it supports my contention that epinephrin is often used in excessive doses.

*Sensitivity*—Subsequently the patient was tested, in the Allergy Clinic at the Los Angeles General Hospital, with a large series of food, epidermal, pollen, and other allergens, but no reactions were obtained. She had been tested several years previously and was told that she reacted to feathers andorris root.

#### DISCUSSION

The ancestry of these patients has been given to show that they are not members of any of the races especially noted for emotional instability.

A traumatic shock is not infrequently the actual or suspected cause of the onset of asthma. In Case 1 the history of such an onset was one of the useful points in determining the line of treatment.

These patients are typical of many others that might be quoted and they demonstrate that the asthmatic may develop the habit of taking large amounts of epinephrin at frequent intervals even though he claims little relief from the drug. The periodicity of injection is often still more. It is a common experience that relatively small doses will be effective when given by the physician but larger ones administered by the patient or family will fail to give relief.

The misuse of epinephrin is often due to the faulty instruction given by the doctor who first advises the "self medication." The pharmacologic axiom that one should adjust dosage so as to obtain the maximum therapeutic effect and the minimum toxic response with a minimum of the drug seems to be disregarded in many instances. In the chronically ill patient who may require a certain drug over a period of years it behooves us to titrate the drug against the patient instead of determining dosage by a fixed rule. Many times the initial dose of epinephrin recommended is 10 to 12 or even 15 minims. Some of these cases might be completely relieved by 2 to 4 minims and perhaps would not need to have this increased for six months or a year. Any marked increase should be only on advice of the physician. The substitution of another drug for a short time and then a return to epinephrin will frequently defer the increase in dosage for another long period.

The earlier writers recognized and possibly overemphasized the neurotic element in bronchial asthma. In more recent times this phase of the problem has too frequently been neglected in favor of specific hypersensitive phenomena which are assumed by some to explain all the signs and symptoms in these patients. The observations reported herein emphasize the need for a thorough study of each individual patient from not one but all the known avenues of approach. The history as in these cases will frequently yield a more important clue than will an exhaustive series of skin tests. The thesis that psychic factors are responsible in large part for the habitual use of epinephrin is supported by the results obtained with psychic therapy. Even in the presence of a considerable degree of emphysema, as in Case 2 this form of treatment may be substituted for epinephrin.

#### SUMMARY

- 1 Many asthmatics use relatively large and frequently repeated doses of epinephrin when self medication is permitted.
- 2 Initial dosage of epinephrin should be determined for each patient and should be the minimum that will give the desired result.
- 3 The time at which the injections are given may show a periodicity which cannot be correlated with climate or other extrinsic factors nor with severity of the attacks.
- 4 When the mental faculties are interestingly occupied the critical periods may be passed without the use of epinephrin or even the development of clinical symptoms.
- 5 It is suggested that this is a form of drug addiction.

6 The nontoxic element is too frequently disregarded and may be more important than specific phenomena. Asthmatic attacks may create a situation that is quite gratifying to the patient.

7 Psychic therapy and the use of a placebo are valuable aids in the control of epinephrin addiction.

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## THE ELIMINATION OF DYES BY THE UTERINE MUCOSA\*

By JOSEPH L. MEYER, M.S., M.D., CHICAGO, ILL.

IT HAS been known for many years that glands which have an external secretion may also eliminate various substances injected into the blood stream or absorbed through the intestinal tract. The substances, however, are not eliminated in the same proportion by all organs. In fact, some organs may eliminate practically all of one substance and not any of another even though the substances may be related closely chemically. Phenolsulphonephthalein,<sup>1</sup> for instance, is eliminated almost entirely by the kidney. Phenoltetrachlorophthalein<sup>2</sup> on the other hand is eliminated almost entirely by the liver. Neither of these substances is eliminated by the stomach. Ivy and Dawson<sup>3</sup> studying the elimination of dyes by the stomach mucosa found that of 33 dyes used, only 13 were eliminated in the gastric juice. Crandall<sup>4</sup> found that the pancreas eliminated only 10 of 92 dyes used. Neither in the work on the stomach nor in the work on the pancreas could the result be predicted on the basis of the chemical structure of the dye. Recently, Belfield and Rohneck<sup>5</sup> showed that the body of the epididymis of dogs will eliminate methylene blue, mercurochrome, and silver arspenamine. They also state that the semen and some of the sperm heads of two men given pyridium by mouth were colored with the dye. The results of the various investigators show, then, that there is a selective elimination of the dye by the secretory organs.

Many drugs have been used in the treatment of infections of the female genital tract. Gentian violet, mercurochrome, and acriflavine have been employed more for the general blood stream infection than for local effect. Kolmer<sup>6</sup> believes that while these drugs may check and eventually remove the bacteremia, they are without marked effect on the local infection.

Because of the inaccessibility of the parts, and because local treatment is sometimes detrimental, any nontoxic antiseptic drug which would be eliminated in appreciable concentration or altogether by the genital mucosa would be theoretically of great value in the management of infections of the female reproductive organs.

Although the drugs recommended for the treatment of genital infections are many and varied, we could find no reference to work showing the elimination of such drugs by the uterine mucosa, therefore, to determine if there

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might be a rational basis for systemic chemotherapy of local female genital infections, we undertook the problem of determining whether the uterine mucosa would eliminate antiseptic dyes which were injected into the blood stream

#### METHODS

The experiments were performed on dogs. In all cases it was noted whether they were pregnant, nonpregnant, or postpartum. In the first series of experiments, the dogs were being used in the physiologic laboratory and were under ether anesthesia during the period between the injection of the dye and the removal of the uterus. Thirty dogs were used under these conditions. The results were not constant. In some instances, mercurochrome or pyridium would appear in the mucosa and at other times they could not be demonstrated. Because of the discrepancies in the results and because the anesthesia was possibly an abnormal factor, the results of these experiments were discarded.

The results in Table I are from experiments in which the dogs were injected intravenously with the dye, anesthetized four hours later, the uterus removed and examined grossly and microscopically, without additional staining.

The presence of the dye was determined by the appearance of the mucosa after removal of the uterus. In the control animals and in those in which the dye was not eliminated, the uterine mucosa assumed the usual death pallor. In those in which the dye appeared, the mucosa retained the color produced by the dye. The appearance of the uterus 'in situ' could not be used as a guide to the presence or absence of the dye because in some instances, the uterus would be deep purple or bluish and as soon as removed would become pale. In others it would be highly injected and deep red, and if the dye did not come through would immediately turn pale after removal.

TABLE I

DYE USED	DOG	PHYSIOLOGIC STATE OF ENDOMETRIUM	RESULT	REMARKS
Mercurochrome	1	Not pregnant	Positive	
	2	Postpartum	Questionable	Am't too small
	3	Pregnant	Positive	Did not abort
	4	Postpartum	Positive	
	5	Near term	Positive	Delivered
Pyridium	1	Not pregnant	Questionable	Am't too small
	2	Not pregnant	Questionable	Am't too small
	3	Not pregnant	Positive	
	4	Postpartum	Positive	
	5	Early pregnant	Positive	
Gentian Violet	6	Not pregnant	Positive	
	1	Not pregnant	Questionable	Mucosa darker than normal
	2	Not pregnant	Questionable	Mucosa darker than normal
	3	Postpartum	Negative	
Acridine	1	Postpartum	Positive	Deep color
	2	Not pregnant	Positive	Deep color
Methylene Blue	1	Not pregnant	Negative	
	2	Not pregnant	Negative	

The term positive means that the mucosa was stained by the dye and the term negative means that the dye was not detected in the mucosa.

The dyes used were methylene blue, gentian violet, acridine, mercurochrome and pyridium. In all cases an aqueous solution was used and in no

instance did the amount of the dye injected reach the per kilo tolerance of man i.e. 5 mg per kilo of body weight

The solutions were injected slowly into the saphenous vein, without anesthesia

#### RESULTS

The experiments carried out while the dogs were under anesthesia are not included in the table, but they demonstrated that mercurochrome, pyridium, and acriflavine would stain the uterine mucosa. The results on unanesthetized dogs are shown in Table I. The dyes had no objective toxic action on the animals.

#### SUMMARY

Mercurochrome, pyridium and acriflavine when injected intravenously gave a distinct color to the uterine mucosa. The result with gentian violet was questionable. Methylene blue did not color the mucosa. It was impossible to determine whether the dye was in the uterine secretion, because of its scantiness in the nonpregnant state and because of its discoloration in the postpartum state.

The physiologic state of the endometrium did not influence the elimination of the dye.

#### DISCUSSION

Acriflavine<sup>7</sup> and pyridium<sup>8</sup> are said to be bactericidal in dilutions as high as 1:200,000. The value of mercurochrome is questioned. Walker<sup>9</sup> does not believe that mercurochrome in concentrations as high as 1:400 in human blood inhibits the development of staphylococci or streptococci. Young<sup>10</sup> claims good clinical results with mercurochrome used intravenously.

We did not determine the actual concentration or the bactericidal value of the dyes in the uterine mucosa, but intend to do so in future experiments. However, if the degree of color may be used as an indication of the amount of dye present, then mercurochrome and acriflavine reach an appreciable concentration in the uterine mucosa.

In addition to the concentration, the length of time the dyes are in contact with the bacteria is an important factor in inhibiting their growth. The time of appearance and disappearance of the dye was not noted in each of our experiments. We know, however, that the dyes which stained the uterine mucosa appeared there within three hours. In three experiments, in which the uterus were removed twenty-four hours after the injection, the dye was still present. Mercurochrome was used in two of these experiments and pyridium in one. This indicates that the dye may possibly increase its value as a local bactericide.

These experiments were carried out on normal organs and the question arises, will the diseased organ eliminate the dye? This can be answered positively only by experiments on the diseased mucosa.

Imshfeld, Malmgreen and Cheav<sup>11</sup> reported that mercurochrome and acriflavine will penetrate gradually from the blood stream into edematous

tissues, but did not believe the concentration would be sufficient to be bactericidal.

Young<sup>10</sup> reports mercurochrome of value in localized infections of the male genitourinary tract. Belfield and Rolnick<sup>5</sup> state that silver arsphenamine is of great value in some forms of epididymitis.

As shown by Kolmer, ethylhydrocuprein and mercurochrome do not under normal conditions pass into the cerebrospinal fluid but in pneumococcus meningitis traces of the former and in streptococcus meningitis traces of the latter, may be found in the cerebrospinal fluid when the dyes are injected intravenously.

The work of Young<sup>1</sup> and of Leonard<sup>1</sup> shows that the kidney may eliminate dyes in sufficient concentration to be of value in infections of the urinary tract.

It may be possible therefore to find an antiseptic dye which will be eliminated from the general circulation by the uterine mucosa in sufficient concentration to be of value in treatment of local infections of the female genital tract.

#### CONCLUSIONS

The uterine mucosa of dogs will be stained by and may possibly eliminate mercurochrome pyridium and acriflavine.

It is questionable whether or not gentian violet will stain the uterine mucosa. Methylene blue does not stain the mucosa under the experimental conditions used. These findings suggest that there is a rational basis for the study of systemic chemotherapy of local disease of the female genital tract.

I wish to thank Dr. A. C. Ivy for generous assistance in conducting this work.

#### REFERENCES

1. Abel and Rowntree, *J. Pharmacol. & Exper. Therap.* 1: 231, 1909.
2. Abel and Rowntree, *J. Pharmacol. & Exper. Therap.* 1: 231, 1909.
3. Ivy and Dawson, *Am. J. Physiol.* 73: 304, 1925.
4. Grandall, Personal Communication, 1928.
5. Belfield and Rolnick, *J. A. M. A.* 89: 2104, 1927.
6. Kolmer, *Chemotherapy*, 1926, H. B. Saunders and Co., p. 150.
7. Browning and Gulbrinsen, *Brit. J. Exper. Path.* 2: 95, 1931.
8. Merek and Co., Pamphlet, 1927.
9. Walker, *Arch. Path. & Lab. Med.* 1: 199, 1926.
10. Young, *J. A. M. A.* 87: 1366, 1926.
11. Hirschfeld, Malmgreen and Crenvy, *J. Pharmacol. & Exper. Therap.* 24: 459, 1925.
12. Leonard, *J. A. M. A.* 85: 1855, 1925.

# LEUCOCYTOSIS FOLLOWING INTRACRANIAL OPERATIONS\*

BY ANATOLE KOLODNY, M D, PH D, F A C S IOWA CITY, IOWA

ABOUT fifteen years ago de Quervain called attention to the fact that leucocytosis in a pathologic condition of the abdomen is not always a sign of infection, ruptured ectopic pregnancy is associated with a high leucocytosis. Hoessli<sup>1</sup> demonstrated experimentally on animals that intraperitoneal hemorrhage provokes leucocytosis. Other observers emphasized that leucocytosis follows hemorrhage in other serous cavities, such as the pleural and the cranial. In distinction from the so-called "leucocytosis of hemorrhage," i. e., leucocytosis after loss of blood when the maximal height of the leucocytosis is reached only after several days and is of a slight degree, leucocytosis after hemorrhage into a serous cavity is much more pronounced and follows immediately after the hemorrhage.

One of the most comprehensive contributions to this question is by Wright and Livingston.<sup>2</sup> In discussing leucocytosis associated with intracranial hemorrhage, these authors draw their conclusions from findings in patients who at the postmortem examination showed basal fractures of the skull or an old subdural blood clot and from attempts at basal fractures of the skull in dogs. The difficulties of conducting similar experiments and, therefore, their questionable reliability is obvious.

In the following I present the results of a study of the white cell count in a series of eighteen operations on the brain for various noninfectious pathologic conditions, mostly tumors. In all these cases the leucocytes were counted immediately before operation, and then soon after the operation, the first postoperative count having been made an average of four hours after the completion of the operation. Following this the leucocytes were counted daily for eight days. In twelve of these eighteen cases the operation was on the cerebrum and in six on the cerebellum. In two cases one of the lateral ventricles was opened during the operative procedure. In all instances the operation proceeded intracranially.

Leucocytosis followed the operation in all the cases. The average increase of the number of leucocytes soon after the operation was 210 per cent of the preoperative number. On the first postoperative day this excess of leucocytes over their preoperative number was 144 per cent, on the second postoperative day 96 per cent, on the third 70 per cent and on the fourth postoperative day 30 per cent. The number of leucocytes reached the normal limits on the fifth or sixth postoperative day.

The leucocytosis in the two cases in which the lateral ventricle was opened was considerably higher than in the other sixteen cases and persisted longer. On the operative day this excess of leucocytes over the normal was

\*From the Department of Surgery, University of Iowa, Iowa City, Iowa.  
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300 per cent, on the first postoperative day it was 290 per cent, on the second day 200 per cent, on the third day 100 per cent, on the fourth 60 per cent, and on the fifth day 40 per cent. The normal limits were reached on the seventh day (Fig 1)

A similar study of the leucocytes was made in six other cases in which the dura was reached but not opened during the operation. These cases showed a slight increase in the number of leucocytes, never above 25 per cent over the normal. This leucocytosis reached the normal limits within thirty-six hours after the operation.

Along with an increase in number of the leucocytes the latter showed a marked shift toward the polymorphonuclear variety. At the same time, the lymphocytes and large mononucleus dropped in number, the former far more than the latter. This is illustrated in Fig 2. This fact is of interest since

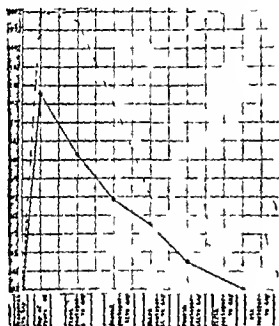


Fig 1

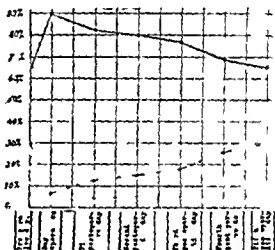


Fig 2

Fig 1—The average increase of leucocytes over the preoperative number is expressed in per cents of the latter. The heavy line shows the average for all eighteen cases, the broken line shows the average for the two cases in which the lateral ventricle was opened during the operation.

Fig 2—This illustrates the differential grouping of the leucocytes prior to and following the operation. The heavy line shows the polymorphonuclears, the broken line shows the lymphocytes and the dotted line the large mononucleus.

it is usually considered that a shift of the differential picture of the leucocytes toward the polymorphonuclears is an indication of an infectious process.

In evaluating the findings of this study the question arises as to the role which anesthesia played in the provocation of the leucocytosis. The influence of anesthesia on the leucocytes has been commented upon by a number of observers. It is thought that ether anesthesia (the anesthesia used in most cases of this study) causes a leucocytosis. Judging from the findings of several observers this ether leucocytosis is never over 70 per cent of the preoperative white cell count. In the six cases in which the dura was not opened during the operation the leucocytosis was never above 25 per cent although all of these cases were operated upon under ether anesthesia.



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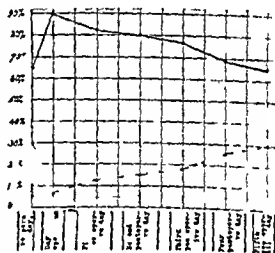


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## CONCLUSIONS

Craniotomies associated with intradural hemorrhage provoke a high leucocytosis. The degree of leucocytic increase is not proportionate to the amount of bleeding but depends upon whether or not the bleeding occurred intradurally or extradurally. When bleeding takes place into a ventricle, the leucocytosis is higher and of longer duration. The leucocytosis seen in craniotomies without opening of the dura is slight, and the leucocytes return to normal in about thirty-six hours after the operation. To depend upon the differential count for a distinction between an infectious and a noninfectious leucocytosis in intracranial operations is erroneous. Postoperative leucocytosis, when the dura is opened or the ventricle entered, usually shows a differential shift toward the polymorphonuclears.

## REFERENCES

- 1 Hoessli, H. Leukocytose bei Intraperitonealblutungen, *Mitt. d. Grenzgeb. d. Med. u. Chir.* 27: 630, 1913-1914.
- 2 Wright, A. M., and Livingston E. M. The Leucocytosis of Internal Hemorrhage, *N. Y. State J. Med.* 23: 286, 1923.

## EFFECT OF SUBCUTANEOUSLY INJECTED EPINEPHRIN IN NORMAL HUMAN SUBJECTS\*

BY THEODORE KOPPANYI, PH.D., SYRACUSE, N. Y.

THE conditions under which subcutaneously injected epinephrin gives a pressor effect in the dog, have been studied by Luckhardt and Koppányi,<sup>1</sup> who found that when the site of injection had been massaged there was a rise in blood pressure of from 15 to 180 mm Hg following each massage. They also showed that in the dog, at least, epinephrin injected underneath the skin remains there for a long time. In spite of numerous pressor effects obtained as a result of the massage of the epinephrinized area, a saline solution extract made nineteen and one-half hours following the injection of 3 cc of epinephrin gave, when injected intravenously, a very marked pressor effect. On the basis of these experiments Lihenthal<sup>2</sup> used this method in the treatment of asthmatic breathing and of surgical shock in man and obtained pressor responses, upon massaging the injected area, for nearly forty-eight hours following the injection of 0.3 cc of epinephrin 1:1000. The blood pressure elevations that he obtained were comparatively slight (from 7 to 15 mm Hg), but he noted very definite therapeutic effects of the drug. Balveat<sup>3</sup> used the method of massaging the injected areas in various allergic diseases (bronchial asthma, asthma complicated with hypertension, urticaria, hay fever) and reported very encouraging results. Peshkin<sup>4</sup> stated that in children with uncomplicated asthma the massage of the epinephrinized areas did not relieve the

\*From the Department of Pharmacology of Syracuse University.  
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asthmatic breathing Dr. A. C. Silberman of the Department of Pediatrics of Syracuse University, on the other hand informs me that in three children (from three to five years old) with uncomplicated asthma (asthmatic bronchitis) the hypodermic injection of 1 cc of epinephrin 1:2600 and subsequent massages of the site of injection repeatedly relieved the asthmatic attacks. He noted however that children desperately object to massage of the injected areas.

There is no doubt that ephedrine is also a very valuable drug in the treatment of allergic diseases and perhaps also in shock but because of the favorable results with epinephrin on the one hand and because of the greater toxicity and relative uncertainty of the action of ephedrine (Halsey<sup>5</sup>) on the other hand one feels justified in stating that ephedrine has not supplanted epinephrin.

In view of these facts it seemed desirable to investigate the action of subcutaneously injected epinephrin in normal human subjects. Such a study might easily establish a connecting link between the animal experiments and the therapeutic application of the drug.

Six young healthy medical students were subjected to subcutaneous epinephrin injections (0.4 cc of a 1:1000 solution). Before injection the normal systolic and diastolic blood pressures and the heart and respiratory rates were determined. From 10 to 15 minutes following the injection of epinephrin there was a rise in systolic blood pressure of 30 to 50 mm Hg without massage. Whereas there was a marked rise in systolic blood pressure, the diastolic pressure generally fell (from 5 to 30 mm Hg). The pulse pressure thus increased and as a rule the heart rate became somewhat slower. Thus vagus beats were unmistakably in evidence. The respiratory rate did not change noticeably.

The injected area was blanched, the face pale, there was a slight muscular twitching in the arms and legs and a thumping palpitating heart was evident (vagus beats). The subjects felt headache, throbbing in the head and ears, some nausea, the mouth was dry and there was in some cases subjective sensation of warmth, in others the hands and feet felt cold. Nervousness and general feeling of anxiety were evident in most subjects.

The injected areas were massaged from fifteen minutes to forty eight hours following injections. In the course of the forty eight hours the area was massaged about 25 times. On the whole, all the symptoms which appeared following epinephrin injections without massage could be duplicated by massage of the injected area. They were however much less marked, the blood pressure rises, e.g. being seldom more than from 7 to 15 mm Hg (in agreement with Lohenthal). The rise was practically the same when one massaged the area twenty minutes or twenty hours following the injection. No definite blood pressure elevations upon massage were obtained after forty eight hours following the injection. In several cases the depot was depleted thirty to forty hours following the first massage.

If we compare these results with those obtained in the dog it seems evident that there is a hemodynamic effect following the hypodermic injection of epinephrin in man even without massage, whereas no such effects could be

produced in the dog. Apparently in man epinephrin is more readily absorbed from the subcutaneous tissues. These results are in close agreement with those published by Clough<sup>6</sup> and by Lyon.<sup>7, 8</sup> These authors reported definite initial rises of the blood pressure (without massage) in man, admitting wide individual variations so far as the magnitude of the response is concerned. We can follow Lyon in emphasizing the fact that epinephrin when given subcutaneously is usually rapidly absorbed, but we have to add that a certain amount of epinephrin is retained and forms a depot beneath the skin. This epinephrin depot yields results for about twenty-four to forty hours following injection.

It is scarcely necessary to state that our subjects did not suffer from any thyroid disturbances, and yet they showed blood pressure elevations following epinephrin injections with or without massage. This fact suggests that the Goetsch<sup>9</sup> test is uncertain as a diagnostic procedure, at least so far as the blood pressure rise is concerned.

It is my pleasant duty to thank all my students who aided me in carrying out this investigation.

#### REFERENCES

- 1 Luckhardt and Koppány, Am J Physiol 81 436, 1927
- 2 Lohenthal J A M A 90 1192, 1928
- 3 Balyent J LAB & CLIN MED 13 1019, 1928
- 4 Peshkin J LAB & CLIN MED 13 1016, 1928
- 5 Hulse, Proc Soc Exper Biol & Med 36 16, 1928
- 6 Clough Johns Hopkins Hosp Bull 31 226, 1920
- 7 Lyon J Exper Med 38 655, 1923
- 8 Lyon Quart J Med 17 19, 1923
- 9 Goetsch Penn Med J 23 431, 1920

# LABORATORY METHODS

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## SOME CHEMICAL ANALYSES OF MEDIUM CULTURED IN LARGE AMOUNTS AND IN SMALL AMOUNTS\*

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WHEN chemical analyses are to be made on a medium on which bacteria are growing a large amount of medium is usually necessary. The medium may be held in a large container from which samples are removed or the medium may be divided into small amounts sufficient for an analysis and the separate amounts given the same inoculation and treatment. Having used small samples kept separately for some chemical analyses of milk cultured with bacteria<sup>1</sup> we thought it would be interesting to find out if there was any significant difference in the results of chemical analyses when the medium was cultured in small samples, or when the medium was kept in one larger container. There are many articles published giving chemical analyses of media kept in either of those ways but we have not seen any article that gives chemical results from one method compared with results from the other method.

For comparative work it was advisable to choose a medium that could be easily measured by pipette for the analyses, so a broth medium was used. A dehydrated dextrose broth was dissolved in distilled water, in the proportion of 13 gm powder to 1 liter water. For the small samples the broth was put into culture tubes one inch by eight inches. For the large amounts a Squibb's pear shaped, separatory funnel of one liter size was used.

The details of handling the medium with the different bacteria varied slightly, but care was taken that the conditions were the same for each bacterium. The separatory funnels and culture tubes were plugged with non-absorbent cotton and sterilized in the autoclave at 15 pounds pressure for twenty minutes on three days. The dehydrated dextrose broth was dissolved in one large container. Eleven to twelve hundred cc of medium were put into each separatory funnel and these were weighed. About 75 cc medium were put into each culture tube and then enough more medium was added to bring medium tube and holder to a definite weight so that the final weight of all tubes with the holder would be the same. The separatory funnels and culture tubes with the medium were sterilized once in the autoclave and then incubated. All samples were incubated at 37° C. The bacteria to be used for inoculation were grown for two days on the same dextrose broth as that used for the analyses. The bacteria used were *B. acidilactici*, *Streptococcus lactis*, and a gram positive spore bearing bacillus resembling in many of its

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TABLE I  
STREPTOCOCCUS LACINUS

	HYDROGEN ION CONCENTRATION ( $P_{H}$ )						TITRATABLE ACIDITY (CC 0.1 N NaOH PER 10 CC MEDIA)						AMMONIA (CC 0.02 N $H_2SO_4$ PER 5 CC MEDIA)					
	LARGE AMOUNTS			SMALL AMOUNTS			LARGE AMOUNTS			SMALL AMOUNTS			LARGE AMOUNTS			SMALL AMOUNTS		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Control	67*	68	68	68	68	67	0.25*	0.31	0.30	0.30	0.29	0.30	0.01*	0.10	0.07	0.04	-0.05	0.04
Control incubated	67	68	68	68	68	68	0.31	0.31	0.31	0.30	0.31	0.30	0.55	0.56	0.41	0.55	0.45	0.51
1 Day	42	42	42	42	42	42	1.13	1.55	1.55	1.57	1.56	1.57						
2 Days	32	32	32	32	32	32	1.83	1.82	1.75	1.90	1.89	1.87						
3 Days	32	32	32	32	32	32	1.90	1.88	1.80	1.97	1.97	1.95	0.61	0.58	0.54	0.56	0.52	0.46
4 Days	32	32	32	32	32	32	1.93	1.90	1.82	1.91	1.93	1.96						
7 Days	32	32	32	32	32	32	1.91	1.83	1.75	1.89	1.89	1.91	0.63	0.56	0.59	0.60	0.49	0.48
31 Days	32	32	32	32	32	32	1.90	1.79	1.75	1.97	2.01	2.00	0.62	0.48	0.48	0.57	0.46	0.42

\*Each result in the table is the average of three determinations.

cultural reactions *Bacillus flexus*. To inoculate with *L. acidilactici* and *Streptococcus lactis* 1 gm. of inoculated broth was added to each tube and 15 gm. of inoculated broth were added to each separator funnel. For the spore bearing bacillus 1 drop inoculated broth was added to each tube and 15 drops of inoculated broth were added to each separator funnel.

To obtain samples for analysis from the separator funnels sterilized water was added to make up for the loss in evaporation until the weight was the same as that of the last weighing, the medium was shaken and an approximate volume of medium let out through the stopcock into another container. Water was added to the medium in the culture tubes until the weight was the same as that before sterilization. Medium cultured in three separator funnels and in three tubes was analyzed at one time and each analysis was done in triplicate.

The chemical analyses made were hydrogen ion concentration, acidity, and ammonia. The hydrogen ion concentration was determined colorimetrically. Standards were prepared using 0.2 M disodium phosphate and 0.1 M citric acid according to the table given by McIlvaine.<sup>1</sup> Mercurous chloride was used to prevent mold growth<sup>4</sup> in the standards and they were kept in a dark cupboard when not in use. The indicators were chosen from those given by Kolthoff<sup>5</sup> and as he suggests made with alcohol when necessary and water. The acidity was determined by titrating 10 cc. medium with 0.1 N NaOH using phenolphthalein as indicator. The method used for the determination of ammonia is that of Van Slyke and Cullen.<sup>17</sup> The ammonia from 5 cc. medium was aspirated into 25 cc. 0.02 N  $\text{H}_2\text{SO}_4$  and the excess acid titrated with 0.02 N NaOH.

The media on which *Streptococcus lactis* and the spore bearing bacillus had been grown when examined bacteriologically at the end of the series of chemical analyses, showed that no contamination had occurred.

The table for *Streptococcus lactis* is given as typical. The figures for the medium cultured in large amounts agree closely. Also the figures for the medium cultured in small amounts are much alike. This agreement indicates that the growths of the bacteria were sufficiently uniform to show no marked differences in their metabolism products when estimated chemically. As the values found for the medium cultured in large amounts agree with those for the medium cultured in small amounts it would seem to make little difference as far as chemical analyses are concerned whether the medium is cultured in one large amount or in separate small amounts.

With these estimations there might be included some work done on milk that was coagulated by a bacterium during growth. This medium is not as easily measured as broth so that the results might show more variation than those for broth. Sugar estimations only were done in the milk series. The method used was taken from Leach *Food Inspection and Analysis*.<sup>8</sup> The milk was measured by pipette and the protein precipitated with Fehling's copper sulphate solution and 0.5 N NaOH according to Soxhlet's method (Leach p. 150). Water was added and the mixture filtered. The filtrate was used with Fehling's solution as given in the Defren O. Sullivan method (Leach p. 294).



The precipitate of cuprous oxide was washed with water, alcohol and ether, dried in the oven, and weighed as cuprous oxide

All these analyses were done on milk kept in small amounts. About 50 cc fat-free milk were put into culture tubes stoppered with nonabsorbent cotton. The volume was marked on the tube and water was added to the mark before the milk was taken for analysis. The first series was done to see how close the agreement would be among milk samples measured from the same tube. Three amounts of milk from one tube were measured into separate containers, the protein precipitated and the mixture filtered. From each filtrate, three separate amounts of filtrate were taken for the cuprous oxide precipitation, the precipitates filtered and weighed separately and the triplicate weights averaged for the results shown in the table. In the second series, milk from different tubes was taken to see if the results would vary more than the results from different samples of milk measured from the same tube. One sample of

TABLE II  
STREPTOCOCCUS LACTICUS

SUGAR IN GM $\text{CuO}$			
SAMPLES FROM SAME TUBE			
	A	B	C
Control	0.1737	0.1741	0.1746
Control	0.1730	0.1736	0.1773
Incubated 3 days	0.1415	0.1459	0.1445
Incubated 4 days	0.1421	0.1419	0.1465
Incubated 5 days	0.1394	0.1374	0.1432
Incubated 6 days	0.1422	0.1319	0.1362
Incubated 12 days	0.1405	0.1371	0.139
Incubated 13 days	0.1397	0.1420	0.1387
Incubated 16 days	0.1329	0.1325	0.1414
Incubated 17 days	0.1342	0.1356	0.1413
Control Incubated 21 days			
Not made to original volume	0.1893	0.1899	0.1898
Control Incubated 22 days			
Not made to original volume	0.1877	0.187	0.1944
Control Incubated 23 days			
Not made to original volume	0.1887	0.1893	0.193

\*Each result in the table is the average of three determinations

TABLE III  
STREPTOCOCCUS LACTICUS

SUGAR IN GM $\text{Cu}_2\text{O}$			
SAMPLES FROM DIFFERENT TUBES			
	A	B	C
Control	0.1825*	0.1858	0.1874
Control	0.1774	0.1842	0.1835
Control	0.1809	0.1852	0.1793
Incubated $\frac{1}{2}$ day	0.1565	0.1518	0.1529
Incubated 2 days	0.1356	0.1461	0.1436
Incubated 5 days	0.1450	0.1367	0.1446
Incubated 6 days	0.1529	0.1531	0.1413
Incubated 15 days	0.1496	0.1494	0.1543
Incubated 16 days	0.1467	0.1488	0.1463
Incubated 19 days	0.1488	0.1399	0.1462
Incubated 20 days	0.1366	0.1422	0.1460
Control Incubated 27 days	0.1748	0.1755	0.1749
Control Incubated 28 days	0.1710	0.1726	0.1709
Control Incubated 29 days	0.1729	0.1779	0.1744

\*Each result in the table is the average of three determinations

milk was measured from three different tubes the protein precipitated and the mixture filtered. From each filtrate, precipitations of cuprous oxide were made in triplicate and the three weights averaged to give the values as reported.

The milk reported in Table II was inoculated with 1 c.c. *Streptococcus lacticus* culture in each tube, and the milk reported in Table III was inoculated with  $\frac{1}{2}$  c.c. *Streptococcus lacticus* culture in each tube. More work was done but as the other tables show similar results only these two are given. As will be seen from the tables the results of the analyses of sugar estimations done by this method when the milk is cultured in separate tubes, do not vary significantly from the results of more than one estimation done on milk from the same container.

### CONCLUSIONS

From the results of these estimations we can conclude that for hydrogen ion concentration, titratable acidity, and ammonia estimations in broth, it makes little difference whether the medium is cultured in large amount and samples removed at various times or whether the medium is cultured in separate containers for each set of estimations. We may also conclude that estimations of sugar in coagulated milk kept in separate amounts under the same conditions do not show any more variation than those in different samples taken from the same container.

I wish to acknowledge my indebtedness to Dr. C. A. Davis who supplied the bacteria and made the bacteriologic examinations.

### REFERENCES

1. Brown, A., Courtney, A. M., Davis, C. A. and MacLachlan, I. F. Etiology of Chronic Intestinal Indigestion. Chemical and Bacteriologic Investigation. *Am. J. Dis. Child.* 30: 603-631, 1925.
2. Kendall, A. I. The Significance and Quantitative Measurement of the Nitrogenous Metabolism of Bacteria. *Studies in Bacterial Metabolism* IV. *J. Infect. Dis.* 30: 211-224, 1925.
3. McIlvaine, T. C. A Buffer Solution for Colorimetric Comparison. *J. Biol. Chem.* 49: 183-186, 1921.
4. Martin, C. J. The Preparation of Sorensen's Phosphate Solutions When the Pure Salts Are Not Available. *Biochem. J.* 14: 98, 1920.
5. Kolthoff, I. M. Indicators, Their Use in Quantitative Analysis and in the Colorimetric Determination of Hydrogen Ion Concentration. Translated by N. H. Furman. 1926. J. Wiley & Sons, New York.
6. Van Slyke, D. D. and Cullen, G. E. A Permanent Preparation of Urease and Its Use in the Determination of Urea. *J. Biol. Chem.* 19: 211-228, 1914.
7. Van Slyke, D. D. and Cullen, G. E. The Determination of Urea by the Urease Method. *J. Biol. Chem.* 24: 117-122, 1916.
8. Food Inspection and Analysis. A. E. Leneh. 1914.

## A NEW METHOD FOR COUNTING BLOOD PLATELETS IN UNDILUTED CITRATED PLASMA\*

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THE blood platelet count and its variations occupy an important position in hematology. The size and extravascular fragility of the platelets however, have impeded the progress of knowledge of their genesis, morphology, function, and number.

Numerous methods for counting blood platelets have been published, but the variations of the "normal number" and the great fluctuation within short intervals indicate that none of the methods has been able to surmount the difficulties of exact enumeration or to eliminate technical errors. In attempts to meet the difficulties few investigators have realized how many and varied are the factors that may serve as sources of error. Obtaining the blood (by skin incision or venipuncture) and actual counting (direct or indirect), usually in a counting chamber do not admit of many variations. The modifications, therefore, of the various methods have chiefly dealt with the diluting fluid.

A great advance in the technique of counting blood platelets was made by the introduction of Oluf Thomsen's<sup>1</sup> macro method in 1919. The new and essential part of this method was the segregation of the blood platelets, advantage being taken of the difference of rate of sedimentation of the platelets and the other blood cells in stabilized blood.

In Thomsen's macro method 4-5 c.c. of blood, obtained by venipuncture, was dropped into a graduated test tube containing 0.5 c.c. of a 10 per cent (or 3 per cent) solution of sodium citrate. After shaking the sample was left for an hour or more for sedimentation. A small amount of the plasma from the surface of the sample (containing platelets) was diluted with physiologic saline in a convenient proportion, usually 1 to 20. The direct counting of the blood platelets was carried out in a Thoma-Zeiss counting chamber after fifteen to thirty minutes. The number of blood platelets in the plasma per c.mm. was calculated from the simultaneous determination of the volume of red blood cells.

This method was quickly introduced in Denmark. It is the chief method in hematology manuals<sup>2, 3</sup> and is still used in many hospitals. It was used in several clinical investigations by Gram,<sup>4</sup> Rud, Kuhnelt,<sup>5</sup> in animal experimentation by Fabricius-Møller,<sup>6</sup> Petri,<sup>8</sup> and both clinically and experimentally by Wittkower.<sup>7</sup>

Unfortunately the method has certain disadvantages limiting its usefulness. As the blood is obtained by venipuncture it is difficult to do the count

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on small children, and it imposes certain limitations on serial examinations. Also, because of the dilution of the serum before filling the counting chamber, the shape, size and number of the blood platelets are altered an important defect in most methods. The method is also useless for serial examination in most laboratory animals except when severe anemia is desired. The error of the method calculated by Meulengracht and Gram was 5 per cent. In ten counts I found it to be 21.9 per cent with diluted plasma (number of platelets 678,400) and 10.4 per cent using undiluted plasma (number of platelets 437,000).

To remedy some of these deficiencies, Oluf Thomsen devised a micro method in 1923<sup>1</sup> based on the same principle. From a skin incision 0.09 cc of blood was obtained by pipette and mixed in a small tube or on a glass slide with the same proportion of sodium citrate as in the micro method. The mixture was drawn into a capillary tube and allowed to stand for sedimentation and the counting done in a Thoma Zeiss chamber ( $\frac{1}{10}$  mm high) on the undiluted or the diluted plasma.

This method was never used in the clinic and to a small extent only in animal experimentation by Anderson<sup>1</sup> on rats and Pettit<sup>2</sup> on rabbits. The percentage of error was calculated by Alb<sup>3</sup> as 5 to 10 per cent and by Pettit<sup>2</sup> as 20.3 per cent.

To make Oluf Thomsen's principle suitable for serial investigations in laboratory animals it was necessary to determine the percentage of error accurately as well as the influence of more or less radical changes in the technique on the blood platelets in the blood sample. The results obtained in testing individual features of Oluf Thomsen's method are applicable to most methods for counting blood platelets previously reported.

Thus when counting platelets it is necessary to distinguish the samples obtained by skin incision from those taken by venipuncture. An admixture of fluid from the tissues can never be avoided in skin incision and this enhances the tendency of the platelets to shrink. This is evident to a certain degree while the blood is flowing, and while it is standing, but especially when the plasma is diluted. The rate of sedimentation of the blood platelets is also diminished and adhesions will occur between the blood platelets and the erythrocytes. In samples obtained from a vein however the size, shape and number of the platelets remain unchanged for a long time.

In the process of dilution preceding the counting, changes in size and shape take place in the platelets especially in the blood obtained by skin incision. The average error therefore is considerably larger in the diluted than in the undiluted plasma.

From a thorough investigation of different salt solutions, fixation fluids, acids etc. it appears that none of the dilution fluids used wholly prevent the shrinkage of the blood platelets. It seems as if the platelets were unable to withstand any appreciable change of the medium in which they are normally suspended. As regards the stabilization medium sodium citrate seems to be the most serviceable.

If blood platelets therefore are counted without considering the points mentioned above the reliability of the count suffers. In Oluf Thomsen's

macro-method the plasma was diluted, in the micro method the blood was obtained by incision. Thus both methods have inherent faults. To count blood platelets accurately one must, (1) simplify method, (2) avoid admixture of tissue juices, (3) stabilize blood properly, (4) avoid dilution, and (5) separate the blood platelets as quickly as possible from the white and red blood cells.

To exclude the most obvious sources of error a new technique, based on the method given by Oluf Thomsen, has been devised, subject to minor modifications. This method is applicable to both man and experimental animals, and is especially suitable for serial examinations (one or more times a day) in rabbits. It is as follows:

1 Draw 0.45 cc of blood from the auricular vein into a 1 cc syringe containing 0.05 cc of 10 per cent sodium citrate. For the puncture, a 2 cm long, medium sized needle cut straight transversely, is used. Both syringe and needle are impregnated with liquid paraffin, and the excess squirted out before the sodium citrate is drawn into the syringe.

2 Transfer blood from syringe into small paraffined test tube close with rubber stopper, shake gently a few times then centrifuge for one minute. In a centrifuge with 5000 revolutions a minute, one minute is adequate for a  $\frac{1}{2}$  cc blood sample. The time was divided as follows:  $\frac{1}{4}$  minute half speed,  $\frac{1}{4}$  minute full speed,  $\frac{1}{4}$  minute half speed, and  $\frac{1}{4}$  minute for stopping. Centrifuging takes the place of the sedimentation by standing in Oluf Thomsen's method. It is important however that in centrifuging, the procedure be carried out in the same manner every time. Any centrifuge can be used, it is only necessary once for all to determine the exact time necessary. Because of the great difference in the rate of sedimentation between the erythrocytes and the blood platelets, the erythrocytes will be thrown down from the upper  $\frac{1}{4}$  or  $\frac{1}{2}$  of the sample before the original content of blood platelets has been changed.

3 Remove a small amount of the blood platelet containing citrated plasma with a fine capillary, place it directly on a  $\frac{1}{20}$  mm high Thoma-Zeiss counting chamber, cover tightly with a cover glass. In order to avoid air bubbles, the cover glass is held obliquely when put in place. Avoid contaminating the sample with liquid paraffin which covers the plasma after centrifuging.

4 Allow to stand fifteen minutes for sedimentation. After this time the platelets will be found to be refractive, sharply outlined, uniform, slightly oval, discoid corpuscles of equal size, evenly distributed in the counting chamber.

5 The platelets are counted in 8 small squares. The sum denotes the number of blood platelets per 1 cmm of plasma. It is superfluous and unnecessarily complicated to recalculate the blood platelet figures found in the citrated plasma per cmm of blood.

In handling rabbits it is convenient to confine them to an animal box with the head protruding through a circular opening. The hairs are removed from the ear, the skin is cleaned, and the vessels made prominent by rubbing with

toluol To avoid loss of blood the needle is introduced subcutaneously for about  $\frac{1}{2}$  cm before the vein is punctured At the same time the needle is fixed with a clip, the prongs of which are attached to the ear above and below the puncture The needle is removed quickly after the blood has been obtained, and the puncture is at once closed with the clip Blood may also be obtained by heart puncture but vein puncture is by far the easiest and safest method In a medium sized rabbit the loss of 1 cc of blood daily for ten to twelve days will not bring about any noteworthy change in the erythrocyte and platelet count In smaller animals like guinea pigs, rats, and mice the blood must be obtained by heart puncture, and it is only possible to make serial investigations every second or third day on account of the danger of a hemorrhagic anemia

In man the blood is obtained by the puncture of a vein, in a 5 cc syringe, or directly in a test tube stabilizing the sample with 10 per cent sodium citrate in the proportion of 1 to 10 In small children where vein puncture may be impracticable one may be forced to obtain blood by the earlier less reliable methods i.e. a freely bleeding prick or incision

The accuracy of the 'centrifuging method' without any dilution of the blood has been determined by making six ten counts The percentage of error, when the blood had been taken by vein puncture was 7.3 per cent (minimum 7.1 per cent) per 760 000 platelets and when blood had been taken by skin incision 10.5 per cent (minimum 9.4 per cent) per 360 000 blood platelets

## REFERENCES

- 1 Thomsen O En Metode til direkte Tælling af Blodpladerne i Blodet Hospitalstid 62 161 1919
- Ibid En Mikrometode til Tælling af Blodplader Hospitalstid 66 406 1923 and Acta med. scandinav 58 515 1923
- 2 Muelengracht E and Gram H C Haematologisk Teknik 1921 Copenhagen
- 3 Muelengracht E and Iversen P Klinisk Mikroskopi Kemi og Baktteriologi ed 4 1924, Copenhagen
- 4 Gram H C On the Platelet Count and Bleeding Time in Diseases of the Blood Arch Int Med 25 325 1920
- 5 Rud, E Blodundersøgelser hos Patienter med Cancer celler i ntern under Radiumbehandling Dissertation 1925 Copenhagen
- 6 Kühnel P Haematologiske Studier i Sangerskabet samt under og igennem et længere Tidsrum efter Fødslen Dissertation 1925, Copenhagen Levin & Munksgaard
- 7 Fabricius Möller J Experimentelle Studier over hæmorrhagisk Diathese, fremkaldt ved Røntgenstråler Dissertation 1922 Copenhagen
- 8 Petri S (a) Om Teknikken ved Blodpladetælling ad mod Thom sen ved Dyreforsøg Hospitalstid 67 65 1924 (b) Sur la technique employée pour la numération des hématoblastes d après la méthode de Thomsen dans les recherches expérimentales sur les animaux, Compt rend Soc de biol 90 881 1924
- Ibid (a) Undersøgelser over Thomsens Blodpladetaællemetoder Hospitalstid 33 513 1924 (b) Sur la numération des hématoblastes d après la méthode de Thomsen Compt rend Soc de biol 91 405 1924
- Ibid Investigations Concerning the Origin of the Blood Platelets I Wright's Theory Acta path & micro biol 2 23 1925
- Ibid Investigations Concerning the Origin of Blood Platelets II Acta path & micro biol 2 97 1925
- Ibid Investigations Concerning the Origin of Blood Platelets III Acta path & micro biol 2 277, 1925
- Ibid Investigations Concerning the Origin of Blood Platelets IV Acta path & micro biol 2 357 1925
- Ibid Investigations Concerning the Origin of Blood Platelets V Acta path & micro biol 3 432, 1926
- Ibid Undersøgelser over Wright's Blodpladeteori en teknisk histologisk og experi

mentel Studie (with a French summary), Dissertation, 1926, Copenhagen, Levin & Munksgaard

- 9 Wittkower Klinische und experimentelle Untersuchungen zur Blutplättchenfrage, Ztschr f d ges exper Med 25 7, 1921, and 26 270, 1922
- 10 Andersen, J V Blodplättetelling efter Oluf Thomsen's Mikro metode hos Rotter på A vitaminfri Kost, Biblioth f Læger 116 625, 1924
- 11 Als, E Oluf Thomsen's Mikro metode til Tælling af Blodplader, Hospitalstid 66 425, 1923

## A RAPID METHOD FOR THE PREPARATION OF FECAL DIGESTS SUITABLE FOR USE IN NITROGEN AND MINERAL ANALYSES\*

By GLENNEVAL STARNES PH D, IOWA CITY, IOWA

**A**NALYSES of feces are usually extremely tedious. The use of fecal digests which may be pipetted† greatly increases the speed of an analysis, without decreasing its accuracy. A method for the preparation of such digests has been in use in this laboratory for over a year, and has proved to be efficient. No claim is made for originality in the method, it is merely a time saving combination of methods that has been tested and found useful.

The feces are collected in the usual manner.<sup>1</sup> If only nitrogen and mineral analyses are wanted, the fecal specimens may be put directly into a 2 or 3 liter wide mouthed Erlenmeyer flask containing about 600 to 800 cc of 10 per cent sulphuric acid. The flask is kept in a refrigerator, the successive specimens being added until collection is complete. The whole material is then brought to a boil in the hood adding more acid if necessary, so that the mixture will be thin enough to pour well and pipette readily. The boiling is continued for from five to ten minutes, being careful to avoid too vigorous heating, or foaming will occur. As soon as the solid material is well broken up, and the mixture appears of even consistency the contents of the flask are cooled to room temperature, and transferred to a 1 or 2 liter volumetric flask, pouring the material through a sieve (an ordinary small sieve, such as is used for a tea strainer, 15 to 20 mesh, is suitable). If the strainer is placed in a funnel, which, in turn, is inserted in the neck of the flask the transfer is simple. The solid material collecting on the sieve can be forced through by gentle pressure with a spatula, so that the only residue left on the sieve, after washing is complete, consists of seeds and like material, the weight, in most diets, being so small that it may be disregarded. If the diet contains an unusual amount of undigestible or difficultly digestible material, so that the residue which does not pass through the sieve is too large to be disregarded, the remaining material may be transferred to a Kjeldahl flask and heated with concentrated sulphuric acid until solution takes place. It is not necessary to carry the digestion on to completion, just to the point where solution is com-

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†A sulphuric acid digest of feces used by Dr O H Gachler of the Department of Biochemistry State University of Iowa for nitrogen determination, was described to us in a personal communication.

plete. This second digest is cooled, diluted somewhat, cooled under running water and added to the original material. The boiling flask, sieve and funnel are washed well with distilled water and the washings added to the original mixture, which is then made up to volume and mixed well. The suspension thus formed lasts for sufficient time to enable sampling with a pipette. It may be kept at room temperature without deterioration, but must be thoroughly mixed before sampling. This digest will be referred to as Digest A.

#### NITROGEN AND CHLORIDE

Digest A is used directly for nitrogen and chloride determinations. The amount necessary for a macro Kjeldahl will vary somewhat with the dilution of the digest but we have found that 10 c.c. samples are usually satisfactory. We have used the Kjeldahl-Gunning technique for nitrogen in urine.

If chlorides are to be determined in feces, certain precautions are necessary in the preliminary boiling in order to guard against loss of hydrochloric acid. The boiling flask is connected to a condenser and the distillate collected in dilute alkali. Chloride determinations are run on this distillate and the amount found (usually very small) is added to the total otherwise determined. Ten c.c. samples of Digest A are taken for the chloride determination, using the Wilson and Ball modification of the Van Slyke method for chloride in blood and tissues.<sup>3</sup>

#### CALCIUM PHOSPHORUS AND TOTAL FIXED BASE

Twenty five c.c. samples of Digest A are placed in 250 c.c. Erlenmeyer flasks, 5 c.c. of concentrated sulphuric acid are added and the mixture is heated on a hot plate in the hood until most of the water is driven off. The flasks should be shaken occasionally to prevent bumping. After cooling for a minute, concentrated nitric acid is added 1 to 2 c.c. at a time, heating after each addition until nitric oxide fumes are no longer given off. When all the organic matter is digested, the solution will be clear and colorless or but slightly yellowish. If a crystalline precipitate has formed it is brought into solution with nitric acid and water, keeping the concentration of nitric acid as low as possible. The solution is cooled, transferred quantitatively to a 100 c.c. volumetric flask and made up to volume. This solution (Digest B) is used for the determination of calcium, phosphorus and total fixed base.

*Calcium*.—A modification of the McCrudden method<sup>4</sup> has been used for the determination of calcium in fecal digests. One hundred c.c. of Digest B is pipetted into a 150 c.c. pyrex beaker and the solution neutralized with concentrated ammonium hydroxide, using rosolic acid (0.5 per cent in 95 per cent alcohol) as indicator. Concentrated hydrochloric acid is then added until the color just changes to a definite yellow. Ten c.c. of 2.5 per cent oxalic acid are then added, the solution is boiled for about one minute, then 10 c.c. of 20 per cent sodium acetate are run in drop by drop and the solution allowed to stand several hours or overnight. The liquid after the addition of the acetate should be tan in color, if the yellow color persists more acetate should be added.

The calcium oxalate is filtered off by suction, using Jena glass filter crucibles No. 10G/<7. The beaker and filter are washed twice with dilute am-



ammonium hydroxide solution (20 c.c. of the concentrated reagent per liter), the outside of the crucible is washed off with distilled water, and the crucible returned to the beaker. The precipitate is dissolved in 10 c.c. of approximately normal sulphuric acid (28 c.c. of concentrated sulphuric acid per liter), heated to 90° C. and titrated with 0.02 N permanganate, keeping the temperature between 70 and 90° C., and following the usual technique of permanganate titrations.

#### Calculation —

As 1 c.c. of 0.02 N permanganate is equivalent to 0.4 mg. of calcium, the calculation will be as follows:

$$\text{Titration value} \times \frac{\text{normality of KMnO}_4}{0.02} \times 0.4 \times \frac{\text{Total vol. digest B}}{\text{vol. sample of B}} \times \frac{\text{Total vol. digest A}}{\text{vol. sample of A}}$$

This reduces to

$$\text{Titration value} \times \frac{\text{normality of KMnO}_4}{0.02} \times 0.4 \times \frac{100}{10} \times \frac{\text{Total vol. digest A}}{25}$$

or

$$\text{Titration value} \times \text{normality of KMnO}_4 \times \text{total vol. digest A} \times 8 = \text{Mg. calcium in total stool}$$

All reagents should be tested for calcium and a blank run if necessary. We have found that many samples of so-called c.p. sodium acetate are contaminated with calcium. By making up the solution in 0.5 per cent ammonium oxalate instead of distilled water, and filtering off the resulting precipitate, most of the calcium salts are removed.

**Phosphorus**—The Fiske and Subbarow method\* for inorganic phosphate in urine may be used with Digest B, if the excess acidity is first neutralized. The sample (4 to 10 c.c. depending on the phosphate content) is placed in a 100 c.c. volumetric flask and diluted to about 60 c.c. with distilled water. After the addition of one drop of phenolphthalein, ammonium hydroxide is added just to a faint pink coloration. The determination is then carried out in the usual manner, the acidity of the reagents destroying the pink color.

**Total Fixed Base**—The method of Fiske\* for the determination of total fixed base in urine may be used with Digest B. The digestion with 4N sulphuric acid may be omitted, all organic matter having been removed in the preparation of the digest.

#### THE METHOD AS APPLIED TO THE FECES OF INFANTS AND SMALL CHILDREN

When the diet consists largely of milk, the calcium content of the stool is often so high that a heavy crystalline precipitate of calcium sulphate may form in Digest A. The digest is boiled until the precipitate is coarse, so that the supernatant material can readily be decanted from it. The liquid is decanted into a liter volumetric flask, the precipitate is washed well with several successive portions of distilled water and the washings decanted carefully into the original mixture which is made up to a volume of 1 liter (Digest A). The precipitate is dissolved in dilute nitric acid and made up to a volume of 250 c.c. (Solution C). A mixture of equivalent portions of A and C is then used in the preparation of Digest B, for example, a 100 c.c. portion of the well mixed Digest A is added to 25 c.c. of Solution C and 25 c.c. samples of this mixture are digested with nitric and sulphuric acids, cooled and made up to a volume

of 100 cc. In calculating, 1250 cc. is used as the total volume of all determinations run on Digest B. Nitrogen and chloride determinations are run on Digest A in the manner previously described using 1000 cc. as the total volume.

## REFERENCES

- 1 Hawk, P. B. and Bergeim, O. *Practical Physiological Chemistry* 1926 p. 790
- 2 Wilson, D. W., and Ball, E. G. *J. Biol. Chem.* 78: 1, 1928
- 3 Van Slyke, D. D. *J. Biol. Chem.* 58: 523, 1923
- 4 McCrudden, F. H. *J. Biol. Chem.* 10: 187, 1911
- 5 Fiske, C., and Subbarow, Y. *J. Biol. Chem.* 68: 37, 1925
- 6 Fiske, C. *Ibid.* 51: 55, 1922

## A NEW METHOD FOR CALCULATING COLORIMETRIC RESULTS\*

By J. CLYDE BAILEY, BALTIMORE, MD.

THE method consists of setting the unknown solution instead of the standard. In this way a constant factor will be obtained by which all determinations of the same type can be multiplied. Below is a more detailed account of the method of determining the factor to be used in each case, together with a comparison of the present method.

## BLOOD SUGAR (CONTINUATION OF PREVIOUS)

Using Standard containing 0 mg. Sugar

Present Method

$$\frac{.0 \times 100}{R} = \text{mg. Sugar per 100 cc. blood}$$

New Method

$$\frac{R \times 100}{.0} = \text{mg. per 100 cc. blood}$$

In the new method

20 represents the setting of the unknown

R represents the reading of the standard against the unknown set at .0

100 is the same as in the old method

It can readily be seen that by the old method the reading of the unknown is subject to constant change hence each determination is a new calculation. In the new method since the unknown is set at a definite depth the same figure is used for every determination while the top figure representing the reading of the standard against the unknown changes. Since the figure representing the unknown does remain the same in the new method, the following cancellation may be made, thereby greatly simplifying the calculation.

$$\frac{R \times 100}{20} = \text{mg. Sugar per 100 cc. blood}$$

Therefore 20 will be the constant factor for blood sugar

*Example*—With the standard reading 16.4 against the unknown

$$16.4 \times 5 = 82 \text{ mg per 100 cc blood}$$

Reading of 23.1

$$23.1 \times 5 = 115.5 \text{ mg per 100 cc blood}$$

#### BLOOD NONPROTEIN NITROGEN (FOLIN WU METHOD)

Standard contains 0.15 mg nitrogen

Present Method

$$\frac{20 \times 30}{\text{R of unknown against standard}} = \text{mg NPN per 100 cc blood}$$

New Method

$$\frac{\text{R of standard against unknown} \times \frac{15}{20}}{30} = \text{mg NPN per 100 cc}$$

Since setting the unknown at 20 in this case gives an inconvenient factor, 15 may be used instead

$$\frac{\text{R} \times \frac{2}{15}}{30} = \text{mg NPN per 100 cc blood} \quad \text{Two is the factor to be used}$$

*Example*—A reading of 17.5 for the standard against the unknown would be  $17.5 \times 2 = 35$  per nitrogen per 100 cc blood

#### BLOOD CREATININE (FOLIN WU)

Present method when using work standard

$$\frac{20 \times 15}{\text{R of unknown}} = \text{mg Creatinine per 100 cc blood}$$

New Method as in the NPN calculation against the setting of 15 may be used instead of 20

$$\frac{\text{R of standard} \times \frac{0.1}{15}}{15} = \text{mg Creatinine per 100 cc blood} \quad \text{One tenth is the factor}$$

*Example*—With a reading of 13.3

$$13.3 \times 0.1 = 1.33 \text{ mg Creatinine per 100 cc blood}$$

#### BLOOD URIC ACID (BENEDICT OF FOLIN WU)

Present Calculation

$$\frac{20 \times 4}{\text{R of unknown}} = \text{mg per 100 cc blood}$$

New Method

$$\frac{\text{R of Standard against unknown} \times \frac{0.2}{20}}{4} = \text{mg per 100 cc blood} \quad \text{Factor to be used}$$

is 0.2

#### CONCLUSION

By setting the unknown instead of the Standard in colorimetric determinations a constant factor will be obtained which will save time and trouble in calculations. This method is not only applicable to blood analysis, but to any analysis where the colorimeter is used.

# A RAPID AND CONVENIENT METHOD FOR SKIN TESTING WITH POLLENS\*

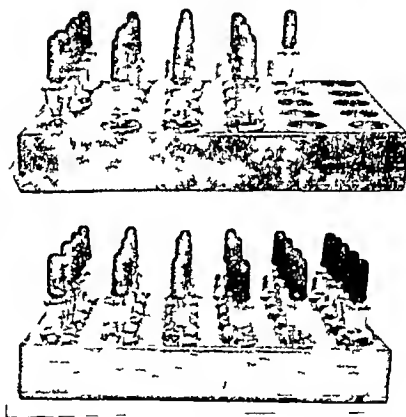
By JOHN P HENRY M.D. MEMPHIS TENN

PRACTICALLY all of the various methods used in performing the 'scratch' tests with pollens are satisfactory as far as determining positive or negative reactions is concerned. Some of these methods however, are more laborious than others and it was a consideration of the time element which led us to adopt the method about to be described.

Two per cent solutions of all the various pollens with which we are concerned in this territory were prepared by extraction in 7 per cent sodium chloride and 46 per cent glycerin as described by Stier and Hollister<sup>1</sup>



Fig 1



Fig

Five cc of each solution was transferred to sterile 15 cc bottles. Ground glass pipettes with heavy rubber nipples keep these bottles tightly stoppered when not in use. The nipples are so adjusted that it is impossible to draw the pollen solution into the rubber portion. (Fig. 1)

Two trays were then devised using heavy boards measuring 11 inches in length, 8 inches in width and  $1\frac{1}{2}$  inches in thickness. This size was adopted to fit our ice box. Thirty holes six rows of five each with a diameter of  $1\frac{1}{8}$  inches were then bored through these boards. Thin boards  $\frac{1}{4}$  inch were then tacked on to act as floors. One tray is painted white and contains

From the Department of Allergy, The Polyclinic, Memphis, Tenn.

our tree pollens. The other tray is of lighter color and contains the grasses and weeds (Fig 2)

The testing bottles are kept in the trays in a definite order. To save further time, mimeographed sheets are used in recording readings. These contain a list of the pollens exactly as they are arranged in the trays. A space is provided opposite each for recording results. This eliminates all writing other than recording "0" for negatives, +, - +, + + +, or + + + + for positives. These sheets are the same size as our history sheets and are filed as a part of the record (Fig 3)

DEPT. OF ALLERGY				THE POLYCLINIC				40 SOUTH DUNLAP ST.				MEMPHIS TENN.			
FOLLIN TEST				NO				DATE				19			
NAME		CUTANEOUS		hr		5 hr		24 hr		hr		5 hr		24 hr	
ROW	1	Johnson Grass								1	JOHNSON GRASS				
I	2	Orris root								1	2 Giant ragweed				
	3	Control								3	Elm				
	4	Blue Grass								4	Blue				
	5	Lamb's quarter								5	spiny asteranth				
	6	Timothy								ROT 1	Timothy				
	7	Spiny asteranth								II	2 Lamb's quarter				
	8	Bermuda								3	Short Ragweed				
	9	Corn								4	Red top				
	10	Sunflower								5	Bermuda				
ROT	1	Red top								ROT 1	ou Ragweed				
II	2	Giant Ragweed								II	2 Joar Retro				
	3	Amar retro								3	Orchard Grass				
	4	Orchard Grass								4	Orris root				
	5	Sheep sorrel								5	Marsh Elder				
	6	Short Ragweed								ROT 1	Control				
	7	Crab Grass								II	2 Crab grass				
	8	Marsh Elder								3	Alfalfa				
	9	N. L. Dock								4	Oats				
	10	Alfalfa								5	Cottonwood				
ROT	1	Cocklebur								REMARKS:					
III	2	N. L. Plantain													
	3	Sou Ragweed													
	4	Cult. Oats													
	5	Annual Sage													
	6	Cat tail													
	7	Sage brush													
	8														
	9														
	10														
ROW	1	Elm													
IV	2	Red oak													
	3	Pecan													
	4	Black walnut													
	5	Horn beam													
	6	Hickory													
	7	Sycamore													
	8	White oak													
	9	Hazel													
	10	Cottonwood													
ROT	1	White ash													
V	2	Birch													
	3	Red cedar													
	4	Sweet gum													
	5	Yellow pine													
	6	Willow													
	7	Cypress													
	8	Box Elder													
	9	Red maple													
	10	Aspen													
ROT	1	Post Oak													
VI	2														
	3														
	4														
	5														
	6														
	7														
	8														
	9														
	10														

FIG 3

When testing a patient, the tests are made in parallel rows of ten extending from the upper portion of the shoulder to the region of the elbow. In this way five rows of ten each can be put on one arm, each row corresponding exactly to two rows on the trays.

After the skin incision has been made, a drop of pollen extract is dropped on the incision, care being taken not to touch the skin in order to avoid con-

tamination of the pipette The solution is then rubbed into the incision with a sterile toothpick This, we believe, offers the simplest and most rapid method possible

#### COMMENT

The only disadvantage which occurs to us is the expense of 5 cc quantities of 2 per cent extracts of each of the pollens for those who test only occasionally We believe there is little danger that the solutions will become contaminated from exposure to air on account of the high salt and glycerin content

The advantages are

- 1 Fifty tests can be performed in twenty minutes at most
- 2 The technic is extremely simple
- 3 No dilution of the extracts occurs as when using the one syringe method of withdrawing solutions from rubber stoppered vials
- 4 Elimination of writing both on skin and records
- 5 While most patients are tested with both trays, it is sometimes desirable to test with trees or grasses and weeds only By separating them into groups, further time is saved

#### REFERENCE

- 1 Stier, Robert F E and Hollister Guy L A Comparative Study of Pollen Antigens as Determined by the Skin Reaction J LAB AND CLIN MED 12 No 12 September 1927

### MICROPHOTOGRAPHIC TISSUE REPORTS\*

BY ROBERT A KILOUFFE MD ATLANTIC CITY N J

IT IS possible that the procedure hereafter described may already be in use but as no report of it has been encountered and those who have seen it regard it as, at least, infrequent, it is described herewith for the benefit of those who may be interested

It consists, in brief, in attaching to the report of histologic tissue examinations embodying the usual gross and microscopic descriptions microphotographs of characteristic fields showing the salient features upon which the histologic diagnosis was made

This procedure has the following advantages

- 1 It assures the pathologist that the clinician or surgeon will see exactly what the pathologist saw and the exact features of the sections upon which the diagnosis was based

It is true that the actual slides are available for study by the clinician or surgeon but it is also true that he may not always come to the laboratory at the most convenient time Moreover unless he is skilled to some degree in the microscopic examination of tissues, the pathologist may not always be sure that they both are seeing the same field the same part of the field or the essential features of the field

The microphotograph, on the other hand, assures all this and permits by means of explanatory legends and corresponding lines upon the print, a clear and unmistakable presentation of the facts

2 In the event that it is desired to secure additional opinions, the slides sent away may be accompanied by prints of the fields deemed of importance

3 A tissue report accompanied by microphotographs is a threefold protection in the case of a dissatisfied patient. It protects the pathologist, the surgeon, and the institution by presenting evidence visible to any one and so explaining and justifying the conduct pursued in handling the case

4 If it is desired to furnish reports to more than one physician concerned in the case, the photographs assure that all will be equally cognizant of the microscopic details of the tissue

5 If a surgeon or clinician desires to present a case or a series of cases at a staff or other meeting, the photographs are not only useful but time-saving as obviating a long drawn out description

6 In the course of time a collection of prints is acquired which are useful in the teaching of interns for example

7 A collection of lantern slides of rare or interesting sections is readily made for case presentation teaching, etc

8 Illustrations for published reports are always available

It may be objected that the method is expensive requires skill and experience in photography and a large amount of apparatus, and hence, is feasible only in large and exceedingly well-equipped laboratories. The purpose of this note in addition to calling attention to the procedure itself, is to emphasize its relative cheapness and its feasibility for even the small hospital and the small laboratory

The amount of skill and experience in microphotography which is required is acquired with surprising ease and rapidity

The question of exposure is, of course of essential importance, but is not as great an obstacle to the inexperienced as it appears at first glance

The proper exposure to secure a clear-cut and satisfactory negative is, obviously influenced by a variety of factors among which may be mentioned the character of the subject to be photographed, the character of the light, the distance from the light and so on

If, however, the camera the microscope, and the light source all bear a constant and unchanged relationship to one another, a very little trial suffices to establish a standard exposure producing satisfactory results and from which minor variations can be made as experience dictates as will be described below

The apparatus used in these laboratories is the Hegener vertical camera manufactured by Zeiss and secured through the A. H. Thomas Company

The camera is mounted on a heavy base, one side of which accommodates the microscope, the position of which is maintained by a fence and clamps. At the opposite end, a self-contained simplified illuminating apparatus is screwed to the sole plate

A tapered column, with a slanting aperture through its plinth for transmission of light to the microscope mirror, is attached to the base between the

microscope and the illuminating apparatus. The vertical motion bar of the camera fits in the bore of the tapered column and is clamped therein by means of a screw. When the latter is released the camera can be swung aside. The eyepiece end of the microscope is then unobstructed so that the object may be viewed under the microscope the illumination centered and other adjustments made which cannot be done satisfactorily while viewing the focusing screen. If desired the camera can be detached from the upright and used otherwise for example on a separate foot without microscope.

A resistance should be used with the lamp in order to provide a means of adjusting the voltage of the lamp terminals exactly to the prescribed value and to reduce the voltage when the full intensity of the lamp is unnecessary as in the preliminary preparation of the object and in photographing at low magnification. Its use extends the life of the lamp.

The list price of the outfit without the microscope is \$155.50. This outfit takes a  $3\frac{1}{4}$  by  $4\frac{1}{4}$  inch picture which is a satisfactory size for the purpose and if the fields photographed are well selected it shows all that is necessary.

The method of procedure is as follows. A satisfactory field is found as usual and the microscope then clamped in position on the camera base or the microscope may be first placed in position the camera swung aside the appropriate field found and the camera then swung into place.

With the shutter open and the ground glass screen in place the light is adjusted by means of the rheostat the diaphragm on the light column and the microscope mirror until the field is evenly and brightly illuminated. The field is then focused. The ground glass screen is then replaced by one of plain glass the light cut down with the rheostat and a finer focus obtained by means of a focusing glass. The shutter is then closed.

A few trials are necessary to secure the standard exposure. If one has had no preliminary experience the simplest method is as follows:

1 Do not in the case of tissues attempt to take a picture with the full intensity of the light.

2 All exposures are time exposures.

3 The brighter the light the shorter the exposure and vice versa.

4 Close the shutter remove the glass screen and insert the plate or film holder.

5 Secure the maximum diminution of light by means of the rheostat and then move the rheostat slide along until about one third the maximum light intensity is obtained or about one third the length of the rheostat.

6 Withdraw the slide and expose the plate or film for an arbitrary number of seconds. Then close the camera shutter push in the slide so as to leave only one fourth the film or plate exposed and again expose for the same time. This procedure is repeated until all four segments of the film or plate have been exposed.

The four segments of the film or plate have thus been exposed for the following times. The whole film or plate received at first two seconds for example one fourth is covered and receives no longer exposure. The three fourths then receives a second exposure of two seconds or four seconds in all. A second fourth of the film or plate is then covered with the slide and



the remaining half again exposed and so on. The exposures of the four segments then are two, four, six, and eight seconds.

When the plate or film is developed, if none of the strips are satisfactory, the procedure is repeated starting with greater or less exposure times as indicated. Once an exposure giving a clear-cut negative is obtained, this point on the rheostat is noted and used, thereafter, for tissue photographs as the "standard exposure time."

The same procedure is followed for oil immersion and high power work as, obviously, the amount of light passing through decreases in proportion to the lens and more illumination or increased exposure or both may be required.

While this crude method will give surprisingly good results in the hands of the inexperienced as experience accumulates minor variations will be indicated in accordance with the character and density of the sections and so on.

As all the sections are stained a color filter is necessary to produce the best results as to contrast.

A very excellent satisfactory and simple filter may be prepared as follows:

Copper sulphate	175 gm
Potassium chromate	17 gm
Sulphuric acid	2 cc
Water to make	500 cc

This solution is placed in the glass cell of the camera. An equally satisfactory color filter for general use is the Wratten K3.

Either plates or film may be used, the Wratten M plate and Eastman panchromatic film being the best.

Developing and printing may be equally simplified and an elaborate dark-room equipment is unnecessary.

In these laboratories because of lack of space a small closet is utilized as a dark room.

It was made light proof with a red light and, as required by the panchromatic film and plate, a No. 3 Wratten safe-light, a fan for ventilation through several small "windows," and a printing machine. Developing and fixing are done in trays. Formulas for these solutions accompany the films and plates or the prepared powders made for x-ray developing may be used.

These come in sealed cans in quantity sufficient to make 1 to 3 gallons of solution and require only to be dissolved in the appropriate amount of water. They are suitable for both negatives and prints.

Once the apparatus is purchased, which may be done for \$200, the expense is negligible. A little more work and a little delay in furnishing the written and illustrated report is entailed but more than compensated for by the results. It is unnecessary to photograph all tissues, only those in which malignancy is found or suspected and not found or those which possess particular interest.

# A SIMPLE DIFFERENTIAL DIAGNOSTIC PROCEDURE IN DISEASES OF THE HEMATOPOIETIC SYSTEM\*

By ARTHUR WEISS B S, M D NEW YORK CITY

## A SIMPLE BUT VALUABLE DIFFERENTIAL DIAGNOSTIC HEMATOLOGIC PROCEDURE

FOR the past four years I have been employing both in the hospital and in my practice a simple device that I have found to be of definite value in the differential diagnosis of hematologic conditions. It consists of a piece of capillary tubing about 3 to 4 inches long that is formed into a U with the bunsen flame.

**Technic** The tip of a finger or the lobe of an ear having been cleansed and dried, is pricked so that it gives an easy steady flow to light pressure. The U tube is held horizontally, one of the arms touching the blood drop. As the blood enters the capillary tube the position of the tube is gradually

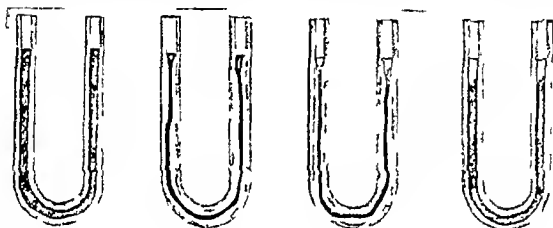


Fig 1

Fig 2

Fig 3

Fig 4

Fig 1—Normal five minutes after filling.

Fig 2—Normal retraction three fourths to one hour after

Fig 3—Retraction in case of severe anemia or infection

Fig 4—No retraction one to twenty four hours after filling as in essential purpura

changed toward the vertical to facilitate the further advance of the blood column until the tube is about three fourths full. One must be careful that no air bubbles enter to interrupt and break the continuity of the blood column. It is very important that the finger prick be sufficiently deep for if the flow is poor the blood will clot before the U tube is filled. When the tube is full it is placed in a vertical position.

**Findings** Normally, it will be found that between three and five minutes the blood will clot and will not move when the position of the tube is altered (Fig 1). In about one half hour the margins of the clot will begin to retract from the sides of the tube, and between one hour and one and a half hours the retraction will be complete as in Fig 2, showing an absolutely clear transparent and colorless serum. From these findings we will then find that the

coagulation time, the bleeding time clot retraction, platelet count, Van den Bergh test and Icteric Index will be normal

Under pathologic conditions, however, we will find the following

1 In *severe infections*, there will be due to an increased globulin content of the blood plasma, a sedimentation of the erythrocytes depending on the severity of the infection (Fig 3). Also due to the increased blood platelets found in severe infections, it will be found that the clot retraction will begin earlier, in fifteen minutes

2 In *hemophilia*, it will be found that instead of the clot being firm in three to five minutes, any altering of the position of the tube will cause the blood column to move. Movement of the blood column can thus be caused after ten to sixty minutes have elapsed (Fig 5)

3 In *suspicious secondary anemias*, depending on their severity, there will be a sedimentation of the erythrocytes as in Fig 3, and early retraction of the blood clot if the anemia is posthemorrhagic. Serum will be clear transparent

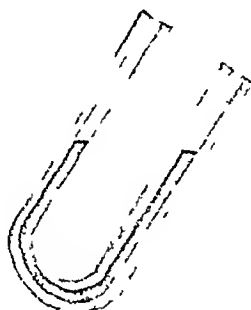


FIG 5—No coagulation five to thirty minutes after filling Hemophilia

and colorless. If the anemia is marked there will be a perceptible increase in the amount of serum as compared with the blood clot, indicating a decreased volume index

4 In the hemolytic anemias, *primary pernicious anemia*, and *hemolytic icterus*, there will be primarily, depending on the severity of the anemia, sedimentation of the red blood cells, secondarily and most outstanding, will be a definitely yellow to golden serum depending on the amount of bilirubin and hematin present in the blood serum. The U tube in a case of obstructive jaundice would also show the same serum color, but in the latter case the history, physical findings, and other laboratory data would definitely place it

5 In *purpura hemorrhagica* (thrombopenic and thrombolytic) the findings are more or less diagnostic. If there was no anemia present, the U-tube at the end of twenty-four hours would look like Fig 1, if anemia were present, it would look like Fig 4. There would be absolutely no retraction of the clot. After splenectomy the U tube will show retraction of the clot returning very quickly. Polycythemia although without retraction can be differentiated by count

**Conclusion** Thus by this simple procedure, necessitating nothing but a piece of capillary tubing and blood gotten by prick of a finger, you can before

having examined cell morphology glean very many useful facts that can act as a guide as to what other tests you should undertake. After examining the cell morphology you can with the aid of the U tube definitely differentiate between a primary or secondary anemia, or a symptomatic or thrombolytic purpura. Where many preoperative cases await hematologic workup, it will be found to be a very useful aid. This procedure does not attempt to supersede the actual taking of the bleeding time, coagulation time, platelet count, and van den Bergh, but it acts as a guide, and also corroborates them.

## A STUDY OF THE MYERS WARDELL METHOD FOR THE DETERMINATION OF CHOLESTEROL\*

BY FRANCES KRASNOW, PH D AND A S ROSEN PHAR D NEW YORK

**I**NQUIRY into the detailed changes of the blood lipins during syphilitic infection necessitated the use of an accurate technic for the determination of cholesterol†. We chose that recommended by Myers and Wardell as the most desirable‡.

However, certain difficulties were encountered. For example, it was found inconvenient to employ a crucible or a beaker for mixing the blood and plaster of Paris. Then the different market plasters varied so that a suitable grade had to be located or a method for standardizing the available ones evolved. Tests during the last four years show that Clover Leaf Plaster X Standard Set, obtained from H B Wiggin's Sons Company, Bloomfield New Jersey, is excellent for the purpose. Moreover the suitability of a brand may be ascertained by comparing it in parallel determinations with  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  (Merk Blue Label) dried to constant weight at  $160^\circ \text{C}$ .

Another source of great concern was the period and temperature for drying the blood plaster mixture, because cholesterol decomposes at high temperatures or even by prolonged heating at low temperatures. Experiments indicate that the safe interval is two hours. If the period is shortened the color develops with a brown tint although there is no loss in cholesterol. Longer periods make for poor color development and are also responsible for low values, the loss varying directly with the amount of heating. Thus for two, four, six, eight, and twenty hours we obtained respectively 186, 167, 151, 137 and 135 mg of cholesterol per 100 cc of whole blood.

Finally, filter paper thumbnails were found more convenient and less expensive than extraction shells. For this purpose an ordinary good grade filter paper (5 inches in diameter) is extracted for six hours with  $\text{CHCl}_3$  in a Soxhlet apparatus and dried thoroughly. It is then folded as shown in Chart I.

The filter paper is folded into form DCE as in figure (a) of Chart I, and a

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†The results of this study will appear in the Archives of Dermatology and Syphilology in a series of articles. They are based on the findings for 99 normal individuals and 60 patients suffering with syphilis.

‡Myers V C and Wardell E L. J Biol 36 14 1918

crease made along AB so that DB is slightly less than BE and CA about  $\frac{2}{3}$  AE, giving flap BEA in figure (b). Then DCAB consists of four layers, DCAB. With the flap facing toward you the farthest layer DCAB is separated from the other three layers, thereby forming a thumb which fits snugly into a glass thumb (2¼ inches high by 1 inch diameter). Instead of suspending the thumb from the condenser, it is set into a Soxlet apparatus. The latter was so made that the ground glass end of its extraction chamber fits inside of the condenser and the siphon tube is not more than 1¼ inches high.

Briefly then, the details of the procedure as adopted are:

The blood (5 cc) is taken into a tube containing the dried residue of 2 cc of K oxalate (1 per cent). If not used immediately, the blood should be kept in the ice chest. The determination ought not to be delayed more than twenty-four hours.

Whole blood (1 cc) is pipetted with an Ostwald-Fohn pipette into a casserole of about 75 cc capacity and covered with 7 gm of plaster of Paris. The mixture is triturated well with a small glass pestle until a uniformly pink powder is obtained. All substance is thoroughly loosened from the sides and

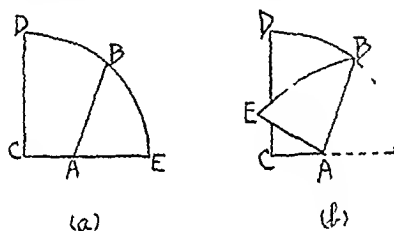


Chart I

bottom with a flexible steel spatula. The dish is placed in the drying oven at  $95^{\circ}$  to  $100^{\circ}$  C for two hours. After cooled, the plaster mixture is transferred quantitatively with the aid of black glazed paper to a fat-free filter paper thumb contained in a glass thumb having several holes in its bottom.

The whole is then placed into the extraction chamber of a Soxlet apparatus of the type described above and set into the extract flask which contains 35 cc of  $\text{CHCl}_3$ \*. Extraction is allowed to proceed for forty-five minutes. The flask containing the extract is removed from the hot plate when the level of the  $\text{CHCl}_3$  in the extraction chamber is one-half the height of the siphon tube. The extract is transferred to a 25 cc volumetric flask. To this are added the washings carried out with the  $\text{CHCl}_3$  which remained in the extraction chamber. After the  $\text{CHCl}_3$  containing the extracted cholesterol is cooled to room temperature or to the temperature at which the apparatus has been standardized, the volume is made up to mark †.

One-tenth cc of  $\text{H}_2\text{SO}_4$  (sp gr 1.84), 2 cc of acetic anhydride (Merck Blue Label)‡ and 5 cc of the extract are pipetted into an ordinary test tube

\*The  $\text{CHCl}_3$  is dried with anhydrous  $\text{Na}_2\text{SO}_4$  or plaster of Paris by shaking several times daily for two weeks and distilled slowly so that the condensed  $\text{CHCl}_3$  enters the receiver drop by drop.

†The  $\text{CHCl}_3$  extract keeps apparently unaltered for a long period when protected from sunlight.

‡The  $\text{H}_2\text{SO}_4$  and acetic anhydride should not be mixed long before the addition of the extract.

(6 inches by  $\frac{3}{4}$  inch), shaken and placed in the dark for ten minutes. The color developed is matched against a naphthol green B standard. A 0.1 per cent solution of this in distilled water serves as the stock concentration. When needed, 1 c.c. of the latter is diluted with 19 c.c. of water\*. If the proper quality of the naphthol green is obtained, this solution set at 15.5 mm. on the colorimeter scale will just match 0.0004 gm. of pure cholesterol in 5 c.c. of  $\text{CHCl}_3$  at 15

## GLYCERIN PRESERVATION OF SEROLOGIC TEST MATERIALS AND THE INFLUENCE OF HEAT ON GLYCERINATED WASSERMANN POSITIVE SERA†

BY FRANCES PERSTEIN AND HAMILTON R. FISHBACK, CHICAGO, ILL.

THE preservation of human sera for future serologic tests and controls is of importance in the laboratory in which the quantity of serum available may be rather small. The question may be still more urgent if the serum is being used for other than routine work.

Of the methods in use for keeping of sera, glycerin preservation<sup>1</sup> seems well adapted for such purpose. Wassermann amboceptor is quite commonly preserved with glycerin. Besides preserving sera for Wassermann and other complement fixation tests it may be used in sera for tests involving agglutinins, precipitins or lysins.<sup>2</sup> We have used it regularly in keeping spinal fluids for the Lange test. The specificity and delicacy of the reaction are not lessened. Also glycerin may be added to colloidal solutions commercially to render them somewhat more stable, and without injuring their reactive qualities.

The glycerin should be of standard chemical purity. It is a stable alcohol and is not altered by long standing. Ordinary glycerin will be found to contain traces of the alkali used in saponification or fatty acids, acrolein or other organic materials. These impurities may be strongly anticomplementary and may tend toward deterioration of the syphilitic reagin in the preserved serum. Sterilization of the glycerin is perhaps desirable theoretically, but whatever bacteria may be carried over in unsterilized glycerin do not grow in the 50 per cent concentration used in preserving sera.<sup>4</sup>

CHART I

EFFECT OF GLYCERIN ON THE SENSITIVENESS OF THE WASSERMANN TEST

CASE NO.	SERUM	0.01	0.02	0.03	0.04	0.0	0.06	0.07	0.08	0.09	0.1 c.c.
49928 D	Non Glycer	0	3	4	4	4	4	4	4	4	4
	Glycer	0	0	4	4	4	4	4	4	4	4
52034 D	Non Glycer	2	3	4	4	4	4	4	4	4	4
	Glycer	0	4	4	4	4	4	4	4	4	4
51635 D	Non Glycer	0	0	2	4	4	4	4	4	4	4
	Glycer	0	0	0	4	4	4	4	4	4	4
51847 D	Non Glycer	0	4	4	4	4	4	4	4	4	4
	Glycer	0	4	4	4	4	4	4	4	4	4

Diluted naphthol green B deteriorate on standing. All matching trials of naphthol do not match the color developed by cholesterol. In emergency it may be used to match against cholesterol.

†From the Pathology Laboratory of Wesley Memorial Hospital, Chicago, and the Department of Pathology, Northwestern University Medical School.

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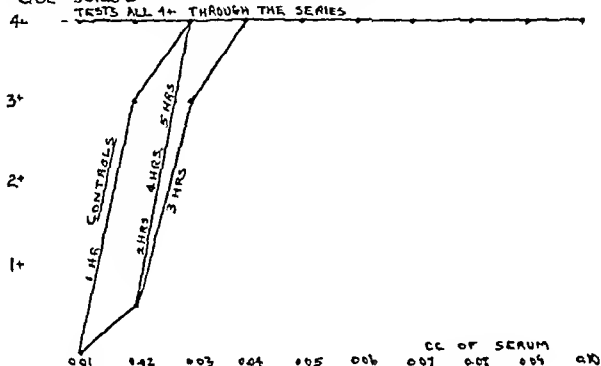
The effect of glycerin upon the Wassermann reacting body is a strengthening one. That it is more than nonspecific absorption of complement is indicated by the persistence of the stronger Wassermann reaction after removal of the anticomplementary activity by titration.

In Chart I, the glycerinated serum is 50 per cent glycerin. The reactions given after the addition of glycerin are seen to be constantly somewhat stronger. One Wassermann modification for which high sensitiveness is claimed<sup>5</sup> has for one of its factors the addition of glycerin. A favorable influence upon a flocculation test for syphilis<sup>6</sup> is also reported.

### II THERMOSTABILITY OF ANTICOMPLEMENTARY ACTION

CASE #50123 D

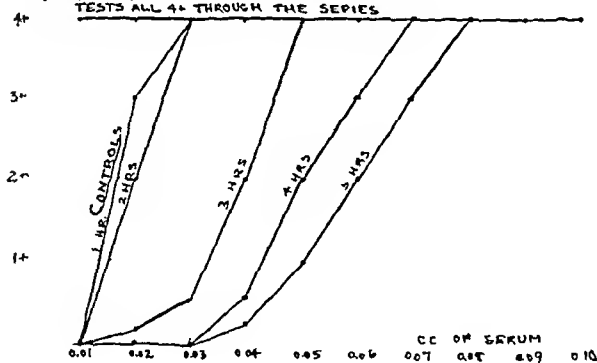
TESTS ALL 4+ THROUGH THE SERIES



### III THERMOSTABILITY OF ANTICOMPLEMENTARY ACTIVITY

CASE #50066 D

TESTS ALL 4+ THROUGH THE SERIES



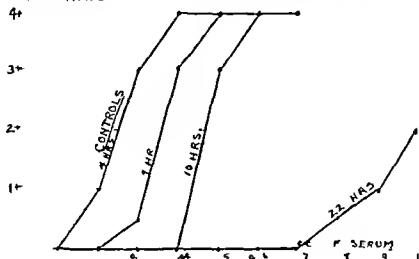
An objection to the use of glycerin as a preservative for Wassermann sera is that it may render them strongly anticomplementary. This effect may be removed from many sera by heating,<sup>1</sup> but returns again on standing. In many others, thirty minutes heating at 56° C weakens but does not remove the anticomplementary effect. It may be also quite heat resistant.

A series of tests was run with prolonged heating of Wassermann positive sera inactivated for thirty minutes at 56° C and preserved by the addition of glycerin to 50 per cent strength, to determine the effect of heat upon those sera with somewhat more thermostable anticomplementary activity. In these tests the serum sample was divided into a number of portions, according to

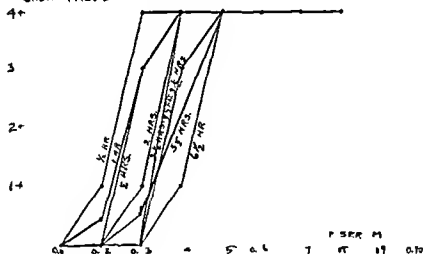
the amount of serum available. Each portion was put into a tightly closed tube, and they were all heated in a closed water bath at 56° C. At the end of each time interval a portion was removed and used at once in the Wassermann titration test.

The results from each separate portion are plotted as to amount of serum and strength of reaction in each tube of the titration series, and the portions are marked according to the length of time of heating. In Charts II and III curves are given for both test and control tubes while in Chart IV only con

#### IV THERMOSTABILITY OF ANTICOMPLEMENTARY ACTION CASE #41171 D



#### V REAGIN THERMOSTABILITY CASE #41128 D



control tube results are plotted. It is noted that the curves for the tests in Charts II and III are identical, since they are 4+ for all ten tubes in each portion. The curves for the control tubes are sometimes superimposed, as in the two hour, four hour, and five hour heated portions of Chart II and it is evident that they all have a common level for the remainder of the curve after the 4+ value is reached.

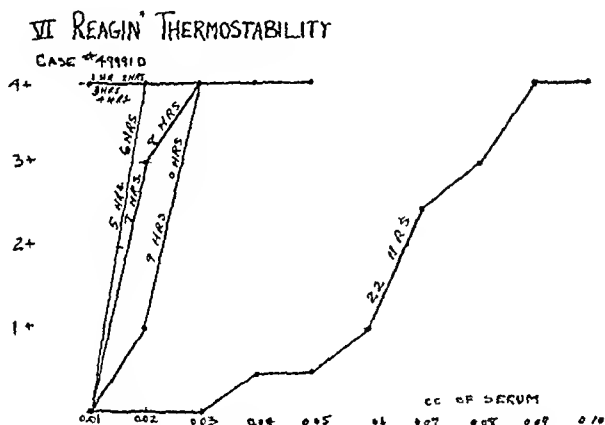
In Charts II, III, and IV, long heating is found to depress the nonspecific complement binding action of these sera very slowly so that complete binding of complement is found with less than 0.05 cc of serum in sera heated varying lengths of time up to ten hours. No evidence is found of destruction of serum components by heat with possible increase of anticomplementary power.



We have found that the method of elimination of anticomplementary activity by titration is effective here also.<sup>7</sup> When the amount is established which is still Wassermann positive and free from anticomplementary action, then this amount may be used in future tests. If the titration is carried low enough there is usually a range of three or more positive tubes without anticomplementary reaction in the controls.

The resistance of the Wassermann reagent to heat was also determined in a series of tests with glycerinated sera. The procedure was similar to that described above.

In Chart V, using a serum of lower Wassermann potency, it will be noted that the values after one hour, and six and one-half hours heating, are only 0.02 c.c. apart, and that a 4+ reaction is given after the final heating by 0.05 c.c. Since the serum samples are 50 per cent glycerinated the amount of serum is actually only 0.025 c.c. In Chart VI, a 4+ reaction is obtained by 0.005 c.c.



serum after four hours of heating, and by 0.045 c.c. after twenty-two hours heating. The decrease in Wassermann titer is gradual, but slight in amount. In Charts II and III also it is seen that all tests remain 4+ through the series, with a total of five hours heating in each serum. The smallest amount used in each titration is 0.005 c.c. of serum.

The amount of heat resistance of the Wassermann reacting body of these glycerinated sera is striking. Other work has been published using a thirty-minute period of heating of glycerinated and nonglycerinated serum, with results slightly in favor of the glycerinated serum, although usually neither gave as strong reactions as the unheated serum.<sup>8</sup> Many workers are agreed that thirty minutes of heating may weaken the Wassermann reaction significantly,<sup>9</sup> although no one seems to have measured such change by the quantitative titration method. Not all sera react alike to heat, and the reasons for such variation are as yet only speculative. If the Wassermann reaction is effected because of a particular physicochemical state of lipoids or lipoid-protein combination in the blood, then glycerol may stabilize such a reactive state as to the influence of both time and heat.

## CONCLUSIONS

Glycerin is a satisfactory preservative for the reacting materials of many serologic tests

The anticomplementary function acquired by many Wassermann positive sera on the addition of glycerin may be quite heat resistant. In such sera the anticomplementary action may be eliminated by titration.

The Wassermann reacting body in glycerinated positive sera was found to survive prolonged heating at 56° C.

## REFERENCES

- 1 Ruediger E H Philippine J Sc Sec B 11 87 1916
- 2 Clock, R O and Beard S D J Infect Dis 21 404 1917
- 3 Moses A Brazil Med 32 40, 1918
- 4 Langworthy V and Wemple M J LAB & CLIN MED 10 864 1924
- 5 Ruediger E H Ann Clin Med 2 23, 1924
- 6 McGlumphy C B Arch Path & Lab Med 2 704 1926
- 7 Fishback H R J LAB & CLIN MED 14 176 1928
- 8 Ruediger E H J Infect Dis 23 102 1918
- 9 Kolmer J A Am J Syph 6 82 1922

## A SIMPLIFIED PORTABLE APPARATUS FOR THE DETERMINATION OF THE CARBON DIOXIDE CAPACITY OF PLASMA\*

By WILLIAM Z FRADKIN, A.B., M.D., BROOKLYN

THE cost and size of the Van Slyke CO<sub>2</sub> apparatus has made it difficult for the general practitioner to use it as a practical clinical aid in the detection of acidosis or alkalosis. Its usefulness has been restricted mainly to hospital laboratories. With this fact in mind I have devised a simpler apparatus which is small, portable, comparatively cheap and easy to handle. It is not intended to replace the present Van Slyke apparatus but rather to fill a need in the general practitioner's laboratory.

## THE APPARATUS

The apparatus as shown in Fig. 1 consists of a 25 cc chamber with two one way stopcocks at the top and side, a 0.5 cc scale on the upper stem divided into 0.01 cc divisions, and a side arm for the reception of plasma and reagents. The bottom of the apparatus is connected by a pressure rubber tube to a leveling cup filled with mercury. The chamber is held in place above and below by screw clamps, the jaws of which are lined with pads of rubber. Capillary (x) is used for the removal of fluids from the apparatus. The entire apparatus is mounted on a heavy metal tube which slides up and down a metal rod fastened to the bottom of the cabinet. The thumb screw in front

\*From the Department of Pathology of the Jewish Hospital of Brooklyn.

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†The apparatus is manufactured by the Emil Greiner Company, Vanlam Street, New York City.

is used to clamp the apparatus at any desired level. In addition to the chamber and leveling cup the cabinet also contains reagent bottles, a pipette, a medicine dropper, and a tube with lubricant.

#### REAGENTS

- 1—12 per cent sulphuric acid
- 2—Caprylic ethyl alcohol
- 3—Distilled water
- 4—Redistilled mercury

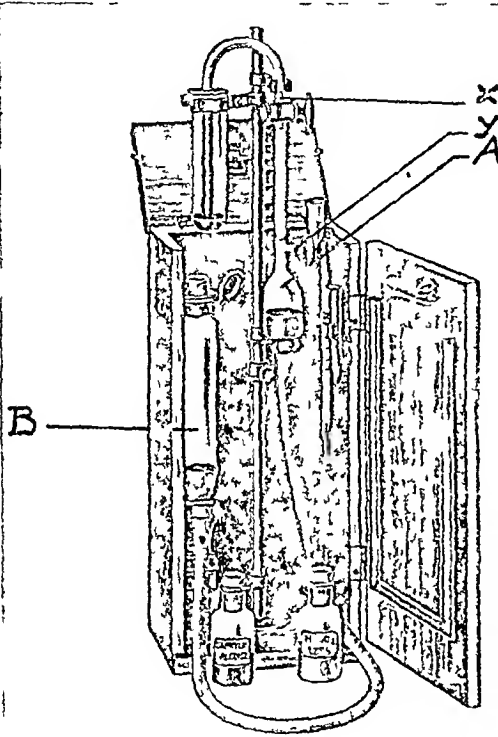


Fig. 1

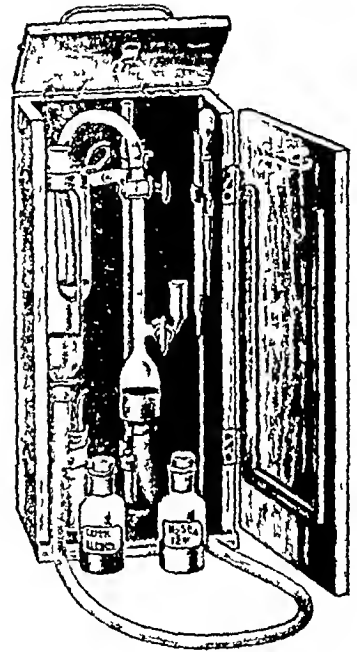


Fig. 2

#### THE DETERMINATION

Raise the apparatus to position (A) by loosening the thumbscrew. Produce a vacuum by lowering the mercury leveling cup. Raise the leveling cup. If the apparatus is tight and gas free the mercury will strike the upper cock with a sharp click. If this click is not heard allow the accumulated gas or fluid to escape by opening the upper cock and repeat lowering and raising the mercury leveling cup until the click is heard. Introduce 0.5 cc of distilled water into the side arm with a pipette. Transfer 0.5 cc of blood plasma into the side arm taking care that the tip of the pipette dips below the surface of the water. With the mercury leveling cup in position (B) rotate stopcock slowly until the fluid in the side arm begins to enter the chamber and displaces the mercury from above. No harm is done if the mercury column in

the chamber is broken by the entrance of the solution. Run in a very small drop of caprylic alcohol into capillary (y). Finally 0.2 cc of 12 per cent sulphuric acid is run in. Then seal the stopcock by running a drop of mercury through the capillary (y). Twelve per cent sulphuric acid is used in order to keep the quantity of fluid to a minimum. Lower the mercury leveling cup until the mercury in the chamber reaches the mark 25. It may be necessary to tap the upper stem with the finger so that all the mercury drops to the bottom. Tip the box gently back and forth for two minutes thus agitating the solution in the chamber. The time of shaking may be controlled by counting slowly 120 numbers which approximates two minutes. The leveling cup with the left hand is then raised rather rapidly so that it reaches the level of the mercury meniscus in the stem. The volume of gas is read at once in order to avoid absorption of the carbon dioxide.

The results are calculated for 100 cc of plasma by using Table IV which is the same as that given by Van Slyke, except that it has been modified to correspond to half the quantity of plasma used.

#### DRAWING BLOOD SAMPLES

Place in an ordinary tube 4 drops of 25 per cent potassium oxalate solution. Puncture a vein with an ordinary Wassermann needle and allow the blood to flow into the tube until about 5 cc is obtained. Cork the tube immediately. Invert the tube two or three times and let the plasma separate by gravity. Then draw off the plasma and analyze. It is not necessary to saturate the plasma with air containing CO if the analysis is done without any delay.

TABLE I

COMPARISON OF CO DETERMINATION WITH THE SIMPLIFIED APPARATUS AND THE VAN SLYKE APPARATUS

Blood samples from patients in acidosis			VOLUME PER CENT DIFFERENCE
	SIMPLIFIED	VAN SLYKE	
1. -----	28.3	30.3	2.0
2. -----	22.6	22.6	0.0
3. -----	18.8	16.8	2.0
4. -----	39.1	38.1	1.0
5. -----	36.1	37.1	1.0
Blood samples from patients without symptoms of acidosis			VOLUME PER CENT DIFFERENCE
	SIMPLIFIED	VAN SLYKE	
1. -----	58.6	57.4	1.4
2. -----	54.5	55.5	1.0
3. -----	64.2	63.2	1.0
4. -----	57.4	55.5	1.9
5. -----	52.6	52.6	0.0

#### DISCUSSION OF TABLE I

Table I shows only slight differences between the two apparatus. Considering the original Van Slyke as the standard, the simplified apparatus is accurate within 1.5 volumes per cent.

CLEANING

The chamber is cleaned with two washings of distilled water, about 5 cc each, admitted through the side arm and expelled through the stem by opening the upper stopcock and raising the leveling cup

LUBRICATION

Both stopcocks must be lubricated occasionally in order that they may turn smoothly and also to avoid any possibility of a leak

TABLE II  
REPRODUCIBILITY OF THE SIMPLIFIED AND VAN SLIKE APPARATUS

On one blood sample from a patient in acidosis		
	SIMPLIFIED	VAN SLIKE
1-----	16.8	17.8
2-----	16.8	16.8
3-----	16.8	17.8
4-----	16.8	15.1
5-----	15.1	15.1
	Average 16.4	Average 16.5
On one blood sample from a patient without acidosis		
	SIMPLIFIED	VAN SLIKE
1-----	57.4	55.5
2-----	56.7	54.5
3-----	56.7	53.6
4-----	55.5	54.5
5-----	54.5	52.6
	Average 56.1	Average 54.1

DISCUSSION OF TABLE II

Table II shows more consistent results in reproducibility with the simplified apparatus than with the Van Slyke

TABLE III  
ANALYSIS OF STANDARD CARBONATE SOLUTION BY THE SIMPLIFIED APPARATUS  
(Solution Contained 52.9 Volumes of CO<sub>2</sub> per 100 cc of Solution by Calculation)

1-----	55.5
2-----	53.6
3-----	55.5
4-----	53.6
5-----	55.5
6-----	53.6
7-----	53.6
8-----	53.6
9-----	54.5
	Average 54.3
	Standard 52.9
	Volume difference 1.4

DISCUSSION OF TABLE III

Table III shows a number of analyses on a standard carbonate solution. The results are within the limit of error of a determination. There is a 2.6 per cent error and only a difference of 1.4 volumes per cent.

TABLE IV

TABLE FOR CALCULATION OF CARBON DIOXIDE CAPACITY OF PLASMA  
(In c.c. of CO<sub>2</sub> Chemically Bound by 100 c.c. of Plasma)

0 100	9 1	0 300	47 5
0 105	10 1	0 305	48 7
0 110	11 0	0 310	49 7
0 115	12 0	0 315	50 7
0 120	13 0	0 320	51 6
0 125	13 0	0 325	52 6
0 130	14 9	0 330	53 6
0 135	15 9	0 335	54 5
0 140	16 8	0 340	55 5
0 145	17 8	0 345	56 7
0 150	18 8	0 350	57 4
0 155	19 7	0 355	58 4
0 160	20 7	0 360	59 4
0 165	21 7	0 365	60 3
0 170	22 6	0 370	61 5
0 175	23 6	0 375	62
0 180	24 6	0 380	63 2
0 185	25 5	0 385	64 2
0 190	26 5	0 390	65 2
0 195	27 5	0 395	66 1
0 200	28 4	0 400	67 1
0 205	29 4	0 405	68 1
0 210	30 3	0 410	69 0
0 215	31 3	0 415	70 0
0 220	32 3	0 420	71 0
0 225	33 2	0 425	71 9
0 230	34 2	0 430	72 9
0 235	35 2	0 435	73 9
0 240	36 1	0 440	74 9
0 245	37 1	0 445	75 8
0 250	38 1	0 450	76 8
0 255	39 1	0 455	77 8
0 260	40 0	0 460	78 7
0 265	41 0	0 465	79 1
0 270	42 0	0 470	80 5
0 275	42 9	0 475	81 6
0 280	43 9	0 480	82 6
0 285	44 9	0 485	83 6
0 290	45 8	0 490	84 5
0 295	46 8	0 495	85 5
		0 500	86 5

## SUMMARY

A simplified portable, cheap and practical apparatus is described for the rapid determination of carbon dioxide capacity of blood plasma. The analysis may be completed at the bedside within a few minutes after the separation of the blood plasma. The results are accurate to within 2.6 per cent of the amount determined.

Further research is being done with this apparatus to make it suitable for other blood chemical tests by the gasometric method.

I wish to express my thanks to Dr Max Lederer, Attending Pathologist for his valuable suggestions and encouragement, also to Mr I. F. Gittleman for checking up the accuracy of the apparatus.

## REFERENCES

1. Van Slyke D. D. and Cullen G. E. *J. Biol. Chem.* 30: 289-290, 1917.
2. Van Slyke D. D. *J. Biol. Chem.* 30: 747, 1917.
3. Van Slyke D. D. and Neil J. M. *J. Biol. Chem.* 61: 551, 1924.

## HINTS TO LABORATORY TECHNICIANS\*

BY AARON E PARSONNET M D, AND JEANNETTE DUPUIS, NEWARK, N J

OF ESPECIAL interest to those who have small laboratories in which every available space must be utilized to good advantage will be this suggestion for holding 50 c c and 100 c c Erlenmeyer flasks

Stout wire hooks such as are used in wardrobes can be screwed into a wall (see Fig 1) each making an ideal rack for two flasks if they are set bottom side up. Hooks were placed about six inches apart one above the other, making a neat and convenient array of flasks.

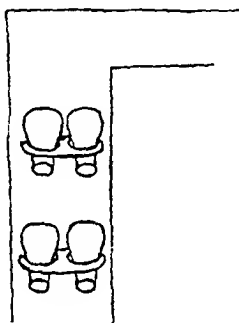


Fig 1

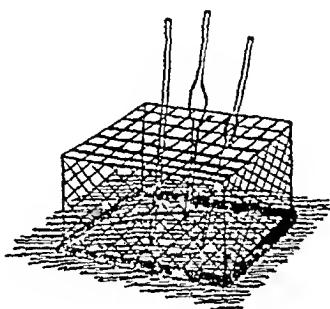


Fig 2

Volumetric pipettes such as those one uses in blood analyses are less apt to break and are more easily kept in order if they are held between the mesh of an inverted test tube basket (see Fig 2). A layer of cotton and gauze may be made for the top of the basket which, when inverted, serves as an ideal pad on which the wet pipettes will drain quickly. In this way one may easily classify pipettes of various capacities.

In the Folin-Wu method of blood analyses a good suggestion is to have made 14 c c and 35 c c volumetric pipettes which may be used for measuring the seven volumes of water in preparing the blood filtrates. In this manner one is certain of accurate measurement and avoids any possible chance of error.

3 MADISON AVENUE

\*From the laboratory of Dr Aaron Parsonnet 3 Madison Ave Newark N J Jeannette Dupuis Technician.

Received for publication October 10 1928

## STUDIES ON ANTIGEN FOR THE KAHN TEST\*

### III THE CORRECTION OF ANTIGEN

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IN A PREVIOUS article from this laboratory<sup>1</sup> it was shown that the standardization of antigen for the Kahn test comprises three steps. The first step is the titration of the antigen, or the determination of the proportion of antigen and salt solution to be employed in preparing the antigen suspension for the serum tests. The second step comprises a determination of the sensitiveness of the antigen as compared with the sensitiveness of standard antigen, comparative tests being carried out with the two antigens, using syphilitic and nonsyphilitic sera. The final step is the correction of oversensitive or undersensitive antigens to the standard degree of sensitiveness. The aim of this article is to correlate the theoretical and practical aspects of antigen correction, and to present in detail the methods for correcting antigens to standard requirements.

#### THEORETICAL ASPECTS OF ANTIGEN CORRECTION

In order to present as clearly as possible the principles of antigen correction, it will be well to summarize briefly some of the facts that we have accumulated regarding antigen. Since the titration process is the first step in the standardization of antigen, we shall first touch upon this phase of the problem, stressing especially the relation between antigen sensitiveness and the relative amount of salt solution used in preparing the antigen suspension.

It will be recalled that the titer of an antigen represents the smallest amount of salt solution which, when mixed with 1 cc. of antigen, results in a suspension that can be dispersed by additional salt solution or by negative serum, forming a clear opalescent solution. During our early studies on the Kahn test, we believed that the inherent differences in the lipid concentration of different antigens would automatically become neutralized in the antigen salt solution suspensions by corresponding variations in the titer and that titration would thus be the only necessary procedure in the standardization of antigen. This view was prompted by the observation that low lipid concentration is usually associated with a low titer and that vice versa highly concentrated antigens have a relatively high titer. It was assumed that uniformity in the concentration of lipids in the suspension would induce uniformity in sensitiveness in the serum reaction, and it was further assumed in the early studies that dilution with salt solution would in general affect the antigen somewhat similarly to dilution with alcohol. Thus for example it was supposed that if Antigen A had a titer of 1 + 14 and Antigen B a titer of 1 + 07 Antigen A, which was richer in lipids than Antigen B would when mixed

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with salt solution at its relatively high titer, be closely parallel in sensitiveness to Antigen B at its relatively low titer. Experience with a large number of antigens indicates, however, that the titer cannot be depended upon as an automatic corrective of antigen sensitiveness. In the supposed case of Antigens A and B, for example, strict adherence to their respective titers may not neutralize basic differences in sensitiveness. It is this fact that necessitated the evolution of several methods of antigen correction.

It is of interest to note that the titer of an antigen does not invariably increase with an increase in lipid concentration. Indeed if the concentration of an antigen is increased by means of an additional amount of its own lipids (see page 988) the amount of salt solution required for the titer tends in some cases to be reduced. For example, if an antigen originally has a titer of  $1 - 14$  the titer might be  $1 + 12$  after the antigen has been concentrated by 10 or 20 per cent.

Let us consider the effects of varying the amounts of salt solution used in preparing the antigen suspension. If the amount of salt solution used is 0.1 or 0.2 c.c. less than that indicated by the titer, the particles of the suspension will not be completely dispersed by the addition of a relative excess of salt solution or nonsyphilitic serum and the mixture of suspension and salt solution or of suspension and negative serum will contain visible particles or will appear turbid due to the presence of particles that are just below the visible range. Inasmuch as the conditions of the test demand that the negative reactions appear clear and opalescent, the use of less salt solution than that indicated by the titer is thus precluded. If, on the other hand, a series of antigen suspensions is prepared with amounts of salt solution progressively greater than that indicated by the titer, these suspensions, which give progressively clearer opalescent solutions when mixed with salt solution or with negative serum, will also be progressively less and less sensitive when tested with syphilitic serum. In other words, an antigen suspension is not fit for use in the Kahn test when prepared with less salt solution than that indicated by the titer and it is more sensitive when prepared according to the titer than when prepared with an amount of salt solution greater than that required by the titer. This latter relation provides a method for the correction of antigens that are more sensitive than standard. Thus, if the titer of the more sensitive antigen is, let us say,  $1 + 12$ , a suspension prepared by mixing 1 c.c. antigen with 15 c.c. salt solution might be sufficiently reduced in sensitiveness to be comparable with standard antigen. This method of correcting oversensitive antigens appears to be less readily controlled than certain other methods to be discussed presently and the method has not been adopted in this laboratory excepting for antigens that are but very slightly oversensitive, which may be brought to standard sensitiveness by using only 0.1 or 0.2 c.c. more salt solution than that indicated by the titer.

It has been shown elsewhere<sup>3</sup> that the sensitiveness of an antigen is closely related to its lipid concentration. An antigen shows maximum sensitiveness only at a certain concentration, referred to as optimum for that particular antigen. If when an antigen is at its optimum concentration the concentration is either increased (by the addition of lipids from the same antigen) or decreased (by dilution of the cholesterolized antigen with cholesterolized

alcohol), the sensitiveness of the antigen is correspondingly decreased. When the increase or decrease in concentration is excessive, antigenic sensitiveness may be reduced to zero.

Our experiments indicate that this relation between lipid concentration and sensitiveness applies to all antigens prepared according to standard requirements, irrespective of whether the source of the lipids is beef, sheep, or pig heart. It has been found, however, that the actual value of the maximum sensitiveness and of the optimum concentration varies for different antigens. At one time we believed that under-sensitiveness in antigens was invariably due to deficient concentration of lipids and could always be corrected by increasing the concentration of the antigen with its own lipids. When this method of correcting under-sensitive antigens is attempted, however, the sensitiveness of the antigen is found to be almost always still further decreased. This would indicate that some other factor besides total lipid concentration controls antigen sensitiveness and suggests that we are probably dealing with at least two types of lipid substances in antigens: an antigenic substance and a substance inhibitory to precipitation. In other words, quality as well as quantity of heart muscle extractives determines antigen sensitiveness. Thus in the collection of antigens we must keep in mind the two variables, ratio of antigenic to inhibitory lipids initially extracted by the alcohol in the preparation of a given antigen and total concentration of heart muscle extractives. Should the original extract contain a favorable ratio, the antigen will be likely to have a relatively high sensitiveness unless its total concentration is too high or too low. If on the other hand the ratio of antigenic to inhibitory substances extracted in a given case is unfavorable, the sensitiveness of the antigen is apt to be low originally, and to become still lower if to the antigen is added an additional amount of its own lipids. It is of interest to note that the sensitiveness of an inherently under-sensitive antigen may be considerably increased by adding to the antigen lipids from a more inherently sensitive antigen.

These relationships are graphically represented in Chart 1, in which the concentration of heart muscle extractives in two representative antigens is plotted against the sensitiveness of the antigens in the Kahn reaction\*. The degree of sensitiveness of standard antigen in the units chosen is represented by the ordinate of the dotted line ST. AXBCDYE is the sensitiveness concentration curve for an antigen having a favorable ratio of antigenic to non-antigenic or inhibitory lipids. If when this antigen is tested its total concentration of heart muscle lipids is equal to (b) or (c) or (d) units (the concentration corresponding respectively to points B, C and D on the curve) the antigen would show more sensitive reactions than standard. If the concentration of the antigen is equal to (x) or (y) units corresponding to points X and Y at which the curve intersects the standard sensitiveness line, the antigen would be equal in sensitiveness to standard antigen. In other words

A precise definition of the units of concentration and sensitiveness chosen is not essential to an understanding of the problem. In general the concentration may be expressed as weight of heart muscle extractives in unit volume of antigen and the sensitiveness in terms of Kahn units, all the tests being carried out with a single lot of individual or of pooled syphilitic serum.

there are two concentrations at which an antigen of this type will correspond in sensitiveness to standard. At the concentrations (a) and (c) the antigen will be undersensitive.

This curve immediately suggests methods of correction for an antigen of this type. If the antigen is oversensitive [the initial concentration of the antigen being represented by (b), (c) or (d)], the concentration may be for purposes of correction either decreased to (x) by dilution of the cholesterolized antigen\* with cholesterolized alcohol, or may be increased to (y) by the addition of some lipid residue from the same antigen. If the antigen proves to be undersensitive, the method of correction must be selected by trial and error to suit the particular case, correction from the point A to X (or to Y) can take place only by an increase in concentration [as from (a) to (x)], correction from the point E to Y (or to X) can take place only by decreasing the concentration, that is to say by dilution.

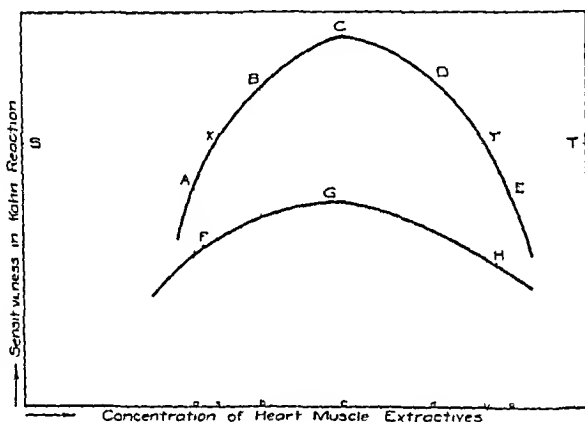


Chart 1

The curve FGH represents the sensitiveness-concentration relation for an antigen with an unfavorable ratio of antigenic to inhibitory lipids. No matter what its total initial concentration may have been, it cannot be corrected to standard either by increasing or by decreasing the concentration of its own lipids, since its sensitiveness-concentration curve never cuts the standard sensitiveness line ST. The correction of an antigen of this type necessitates the addition of lipids from some other source, as for example from sensitizing reagent<sup>4</sup>.

The practical conclusions that may be drawn from these curves may be summarized as follows:

If an antigen is initially *oversensitive*, it may be corrected either by (a) diluting it with cholesterolized alcohol or by (b) increasing the concentration of its own lipids. In general the former method is to be recommended as simpler and more economical. Experience with a large number of antigens received for standardization at this laboratory has indicated, however, that the

\*The concentration referred to in this discussion and in the chart is that of heart muscle extractives and does not include the constant 0.6 per cent of cholesterol which is always added to antigens used in the Kahn test.

sensitiveness of many oversensitive antigens is not readily decreased by a moderate amount of dilution (representing a decrease in lipid concentration of from 10 to 25 per cent), but may on the other hand, be decreased to standard by from 10 to 25 per cent increase in concentration. In such cases, in which we are obviously dealing with the CY portion of the AXBCDYE curve the method of concentration is to be recommended, since that method of correction is always chosen which embodies the least modification of the antigen. Some oversensitive antigens may also be corrected by (c) increasing the amount of salt solution used in preparing the antigen suspension beyond the requirement of the titer.

If the antigen is *undersensitive*, the deficiency in sensitiveness may be due to an excess of lipids even with a favorable ratio (point E on curve AXBCDYE). In this case the antigen may be corrected by dilution with cholesterolized alcohol until the concentration and sensitiveness correspond to the point Y. Theoretically an antigen might be undersensitive in spite of a satisfactory ratio of its lipid types, if as initially prepared, it is too dilute (point A on curve AXBCDYE). In practice, however, it is found that the standard method of preparation rarely or never yields an antigen which before correction combines a favorable lipid ratio with a meager total lipid content. Therefore if an antigen is undersensitive concentration of its own lipids is never used as a method of correction. There remains the possibility that the antigen is undersensitive due to an unfavorable ratio of its lipid types as illustrated by curve FGH. In a case of this sort neither dilution with alcohol on the one hand, nor an increase in the concentration of the same type of lipid on the other hand will result in an adequate increase in sensitiveness and the correction must be made by adding to the antigen some sensitizing lipids from a different source. If sensitizing reagent is added the amount required will in no case exceed about 2 or 3 parts per 100 parts of antigen\*. Thus, although sensitizing reagent is about twice as concentrated as most antigens the correction of an antigen by the addition of sensitizing reagent affects the total lipid content of the antigen to a very slight extent. The effect of adding sensitizing reagent to a newly prepared antigen can usually be intensified by diluting with cholesterolized alcohol the mixture of antigen and sensitizing reagent. Therefore if an antigen is undersensitive (a) the method of dilution is first attempted, should this method prove unsuccessful the antigen is corrected by (b) the addition of sensitizing reagent alone or by (c) the addition of sensitizing reagent and cholesterolized alcohol.

#### PRACTICAL ASPECTS OF ANTIGEN CORRECTION

*Reagents Used in Antigen Correction* Two reagents are used in the correction of antigen, cholesterolized alcohol and sensitizing reagent. The former is prepared by dissolving 0.6 gm cholesterol in 100 cc of 95 per cent alcohol as described in previous publications. A complete account of the preparation of sensitizing reagent is given in a previous article of this series\*.

*Calculations Used in Antigen Correction* 1 Addition of Sensitizing Reagent The amount of sensitizing reagent added to antigen is expressed in terms of the number of parts of reagent added to 100 parts of uncorrected antigen, or, more briefly, and quite accurately for the low proportions of reagent used for antigen correction, as the per cent of reagent present in the corrected antigen. For example to correct an antigen with 15 per cent sensitizing reagent, 15 c.c. of this reagent are added to 100 c.c. of antigen.

2 Method of Concentration In concentrating an antigen with its own lipids, the increase in concentration is also interpreted on a percentage basis. Thus a 25 per cent increase in concentration is brought about by evaporating to dryness 25 c.c. of the antigen (noncholesterolized) and dissolving the residue in 100 c.c. of the cholesterolized antigen, or in general, by dissolving the residue from 0.25 c.c. noncholesterolized antigen in each cubic centimeter of cholesterolized antigen.

A scheme that may be found useful in preliminary tests, when an antigen is to be corrected by the method of concentration of its own heart muscle lipids, is to concentrate, as outlined above, a fraction of the antigen by, let us say, 30 per cent, preparing a sufficient quantity for eight or ten series of ten-serum tests. This fraction may be referred to as Fraction 1. If in one or more series of tests it appears that 30 per cent increase in concentration is excessive (antigen sensitiveness reduced below that of the standard), the remainder of Fraction 1 is used for preparing, by dilution with calculated amounts of cholesterolized alcohol, one or two other fractions of concentrations lower than that of Fraction 1, but respectively greater, by let us say 20 and 10 per cent, than that of the original uncorrected (oversensitive) antigen. Fractions 2 and 3 may then be tested in one or more series of 10 serum tests, and if the results with one of the fractions agree with the standard, a volume of that fraction is prepared from the original antigen by the method of concentration, sufficient for three-tube tests with at least 20 sera, for one-tube tests with at least 100 sera, and for several quantitative tests\*. In preparing Fraction 2 (and similarly Fraction 3) from Fraction 1 for the preliminary tests, the amount of alcohol to be added to each cubic centimeter of Fraction 1 may be calculated from the formula

$$V = \frac{P_{c_1} - P_{c_2}}{100 + P_{c_2}}$$

where  $V$  = number of c.c. cholesterolized alcohol to be added to 1 c.c. of Fraction 1,  $P_{c_1}$  = per cent increase in concentration of Fraction 1 over the concentration of the original antigen, and  $P_{c_2}$  = the per cent increase in concentration (again over the concentration of the original antigen) of each fraction that is to be prepared from Fraction 1. For example, to prepare two fractions (2 and 3) respectively 20 and 10 per cent more concentrated than the original, from 8 c.c. of a fraction (Fraction 1) that is 30 per cent more

\*In this laboratory antigens are not reported as standard until they have shown comparable results with the standard in several 10 serum series of 3 tube tests in 1 tube tests with at least 100 sera and in quantitative tests with two or more sera.

concentrated than the original, we should proceed as follows. For the preparation of Fraction 2 we must add  $\left(\frac{30 - 20}{100 + 20}\right) = \frac{1}{12} = 0.083$  cc cholesterolized alcohol to each cc of Fraction 1, or  $4 \times 0.083$  cc = 0.33 cc cholesterolized alcohol to 4 cc of Fraction 1. Similarly Fraction 3 is prepared by adding  $\left(4 \times \frac{30 - 10}{100 + 10}\right) = 0.72$  cc cholesterolized alcohol to 4 cc of Fraction 1.

3 Method of Dilution. The correction of an antigen by adding cholesterolized alcohol is referred to in terms of a percentage decrease in lipid concentration of the antigen. For brevity in tables, the term percentage dilution is employed, but it must be stressed that this percentage does not coincide in value with the number of parts of alcohol added to 100 parts of antigen. Suppose it is desired to decrease the concentration of the lipids in a given antigen by 20 per cent. If the original concentration was 2 gm per 100 cc the concentration after correction will be 2 minus 20 per cent of 2 or  $2 - \left(\frac{20}{100} \times 2\right) = 1.6$  gm per 100 cc.

In other words we must add to the antigen not 20 cc alcohol per 100 cc of antigen but sufficient alcohol to reduce the lipid concentration of the antigen from 2 gm per 100 cc to 1.6 gm per 100 cc. The relation between the desired per cent decrease in concentration and the volume of alcohol to be added is given in Table I. The figures at the right refer to the number of cubic centimeters of cholesterolized alcohol to be added to 1 cc antigen while the column at the left gives the corresponding per cent decrease in the concentration of the antigen after dilution. To continue our previous illustration suppose that we wish to reduce the concentration of 45 cc antigen by 20 per cent. According to the table we must add to each cubic centimeter of antigen 0.25 cc cholesterolized alcohol and thus to 45 cc antigen we add  $45 \times 0.25 = 11.25$  cc cholesterolized alcohol.

In case it is desired to decrease the concentration of an antigen by a per cent not listed in the table the following formula may be employed:

$$V = \frac{P_d}{100 - P_d}$$

where  $P_d$  = the desired per cent decrease in antigen concentration and  $V$  = the volume of cholesterolized alcohol (in cc) to be added to each cubic centimeter of cholesterolized antigen. Suppose it is desired to reduce the concentration of 1200 cc cholesterolized antigen by 27 per cent. In this case  $P_d = 27$  therefore  $V = \frac{27}{100 - 27} = \frac{27}{73} = 0.37$  cc cholesterolized alcohol. Thus to 1200 cc cholesterolized antigen we must add  $1200 \times 0.37 = 444$  cc cholesterolized alcohol.

#### PROCEDURES OF ANTIGEN CORRECTION

When a newly prepared antigen has been titrated according to the method described in previous publications and has been found to differ from standard antigen in one or more series of comparative 10 serum tests it is corrected in

the following manner depending on whether it is more sensitive than standard or less sensitive than standard

### 1 *Correction of Antigens More Sensitive Than Standard*

#### (a) *By dilution of the antigen with cholesterolized alcohol*

(1) Ten c c amounts of the more sensitive antigen (cholesterolized) are measured into two 25 c c Erlenmeyer flasks

(2) To one flask is added 11 c c (10 per cent dilution) and to the other 25 c c (20 per cent dilution) of cholesterolized alcohol

(3) The original antigen and the two diluted antigens are tested with 10 sera, most of which are known to give weakly positive reactions, employing standard antigen as a control

(4) Suppose the antigen which has been modified by 20 per cent dilution gives the same results with these sera as standard antigen, this diluted antigen is given a check test with 10 different sera. If the check series shows comparable results, the entire amount of more sensitive antigen is accordingly corrected by 20 per cent dilution with cholesterolized alcohol. (For relative volume of alcohol to be added see Table I.) The diluted antigen is once more compared with the standard in one or more series of tests, and if comparable results are again obtained, the corrected antigen is declared standard.

(5) If neither 10 nor 20 per cent alcohol dilution brings the antigen to standard sensitiveness, but approximates this sensitiveness, different per cent dilutions are tried, if the results are markedly more sensitive than standard even at 25 per cent dilution, then the concentration method is to be used.

TABLE I

DECREASING THE CONCENTRATION OF ANTIGEN BY ADDITION OF CHOLESTEROLIZED ALCOHOL

P <sub>d</sub> DESIRED PER CENT DECREASE IN CONCENTRATION (OR PER CENT DILUTION)	V VOLUME, IN C C OF CHOLESTEROL IZED ALCOHOL TO ADD TO 1 C C ANTIGEN
5	0.05
6	0.06
*7	0.075
8	0.087
9	0.10
*10	0.11
12	0.14
*15	0.18
18	0.22
*20	0.25
25	0.33
30	0.43
33	0.50
35	0.54
40	0.67
45	0.82
50	1.0

\*These per cent dilutions are the most frequently employed in antigen correction

\*See footnote on page 984

Tablo II illustrates the correction of a more sensitive antigen (Antigen I) by dilution with cholesterolized alcohol. In this case the antigen after 30 per cent dilution with cholesterolized alcohol gave results comparable to the standard.

(b) *By concentration of the lipids in the antigen*

(1) Into 2 small evaporating dishes are measured, respectively, 1 and 2 c.c. amounts of the oversensitive antigen in a noncholesterolized state.

(2) With the aid of an electric fan, the two amounts are evaporated to dryness. This takes but a few minutes.

(3) Each of the residues is dissolved in a 10 c.c. amount of cholesterolized antigen, the modified antigens thus formed contain respectively 10 and 20 per cent more extract lipids than the original antigen.

(4) The original antigen and the two modified antigens are tested with 10 sera employing standard antigen as a control.

(5) If the modified antigen in which the increase in concentration of lipids is 20 per cent gives the same results with sera as does standard antigen, the check examinations are repeated with 10 additional sera (to eliminate the possibilities of error) and the entire amount of more sensitive antigen is accordingly concentrated to the extent of 20 per cent. The newly corrected antigen is finally declared standard after a given number of additional comparative tests with standard antigen similar to those described above in the case of correction by dilution.

TABLE II

A MORE SENSITIVE ANTIGEN (ANTIGEN I) CORRECTED TO STANDARD REQUIREMENTS BY DILUTION WITH CHOLESTEROLIZED ALCOHOL

SERUM NO	STANDARD ANTIGEN TITER 1 + 11			ANTIGEN I								
				UNCORRECTED TITER 1 + 165			15% DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 13			30% DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 11		
	1	2	3									
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	±	±	++	+++	-	±	+++	-	-	±
3	-	+	++	±	+++	+++	±	++	+++	-	+	++
4	-	-	-	±	-	++	-	±	±	-	-	-
5	-	±	±	±	++	+++	-	±	+	-	±	±
6	-	-	±	-	±	+++	±	±	++	-	±	±
7	-	±	±	±	++	+++	±	+	+	-	±	±
8	-	+	+	+	+++	+++	±	++	+++	-	+	+
9	-	-	±	±	±	++	-	±	+	-	-	±
10	-	-	-	-	-	±	-	-	±	-	-	-

(6) If neither 10 nor 20 per cent increase in concentration brings the antigen to standard sensitiveness, the results obtained with these concentrations will indicate other concentrations which should be tried.

Table III illustrates the correction of a more sensitive antigen (Antigen I) to standard requirements by increase in concentration. In the case of this antigen, a 25 per cent increase in its lipid concentration was necessary for



correction. An interesting observation regarding this antigen is that its titer before correction was 1 + 165, while its titer after correction was 1 + 13.

It should be noted that Antigen I, corrected by an increase in its lipid concentration (Table III) is the same antigen that was corrected by dilution with alcohol (Table II). The possibility of correcting an oversensitive antigen by two seemingly opposite alternative methods is to be expected from the nature of the relationship between the lipid concentration of an antigen and its sensitiveness. As was indicated in the first part of this article, antigen sensitiveness is reduced by increasing as well as by decreasing its lipid concentration.

TABLE III

A MORE SENSITIVE ANTIGEN (ANTIGEN I) CORRECTED TO STANDARD REQUIREMENTS BY INCREASE IN LIPID CONCENTRATION

SUFFIX NO	ANTIGEN I											
	STANDARD ANTIGEN TITER 1 + 11			UNCORRECTED TITER 1 + 165			LIPID CONCENTRA TION INCREASED 50% TITER 1 + 13			LIPID CONCENTRA TION INCREASED 25% TITER 1 + 13		
	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	±	+	±	+	++	-	-	-	-	±	+
3	±	+	++	++	+++	+++	-	-	±	-	+	++
4	-	±	±	±	+	+++	-	-	±	-	±	±
5	-	±	±	±	++	+++	-	-	±	-	±	+
6	-	±	±	±	++	+++	-	-	-	-	±	±
7	-	±	+	+	++	+++	-	±	±	-	±	+
8	-	±	++	++	+++	+++	-	-	-	±	±	++
9	-	-	±	±	+	++	-	-	-	-	-	±
10	-	-	-	-	-	±	-	-	-	-	-	-

(c) *By increasing the amount of salt solution in the antigen suspension beyond requirement of titer*

(1) We shall assume that the titer of the oversensitive antigen is 1 + 13. Three antigen suspensions are prepared by mixing 1 c c amounts of this antigen with 13, 15, and 17 c c quantities of salt solution.

(2) These three suspensions are tested with sera, employing a suspension prepared from standard antigen as a control. If the suspension prepared with 15 c c salt solution gives results with sera of the same degree of sensitiveness as that of standard antigen, this amount of salt solution (instead of the amount indicated by the titer) might be employed in the preparation of the suspension from this antigen. In this case it is the antigen suspension and not the antigen itself that is modified in order to reduce the sensitiveness to standard requirements.

As in the case of the previous methods of antigen correction this method also requires a large number of comparative tests with standard antigen before the antigen with the modified titer may be declared as standard. Table IV illustrates the correction of a more sensitive antigen (Antigen II) by increasing beyond the titer the amount of salt solution in the antigen suspension.





TABLE VII

A LESS SENSITIVE ANTIOEN (ANTIGEN V) CORRECTED TO STANDARD REQUIREMENTS BY ADDITION OF SENSITIZING REAGENT PLUS DILUTION WITH CHOLESTEROLIZED ALCOHOL

SERUM NO	ANTIOEN V											
	STANDARD ANTIOEN TITER 1+11			UNCORRECTED TITER 1+10			ADDITION OF 0.5 PER CENT SENSITIZING REAGENT PLUS 20 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1+10			ADDITION OF 10 PER CENT SENSITIZING REAGENT PLUS 20 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1+11		
	1	2	3	1	2	3	1	2	3	1	2	3
	1	2	3	1	2	3	1	2	3	1	2	3
1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
3	+++	+++	+++	-	+++	+++	+++	+++	+++	+++	+++	+++
4	+	+++	+++	-	+	+++	+	+++	+++	+	+++	+++
5	+	+++	+++	-	+	+++	+	+++	+++	+	+++	+++
6	±	+++	+++	-	-	+++	-	+++	+++	±	+++	+++
7	±	+++	+++	-	-	+++	-	+++	+++	-	+++	+++
8	-	+	+++	-	-	+++	-	±	+++	-	±	+++
9	-	-	+++	-	-	+++	-	±	+++	-	±	+++
10	-	-	+	-	-	+	-	-	+	-	-	-

## 2 Correction of Antigens Less Sensitive Than Standard

### (a) *By dilution of the antigen with cholesterolized alcohol*

Since antigens may be of low sensitiveness due to excessive concentration of lipids obviously it should be possible to correct certain undersensitive antigens by dilution with cholesterolized alcohol. This method of correction which is carried out as was described for an oversensitive antigen, method (a), is illustrated in Table V (Antigen III)

### (b) *By addition to the antigen of sensitizing reagent*

(1) Ten c c amounts of the undersensitive antigen are measured into two 25 c c Erlenmeyer flasks

(2) To one flask is added 0.05 c c sensitizing reagent while to the other is added 0.1 c c sensitizing reagent

(3) The two modified antigens are tested with sera in the usual manner, employing standard antigen as a control. If one of the modified antigens gives results comparable with standard, the whole amount of antigen is corrected accordingly, and after repeated check tests, is declared standard. In case no parallel results are obtained, other combinations of sensitizing reagent and antigen are tried. It should be noted that the sensitiveness of an antigen is usually increased very markedly when from 2 to 5 parts of sensitizing reagent are added to 100 parts of antigen. When the proportion of sensitizing reagent used exceeds 6 or 7 parts per 100, the precipitates in the serum reaction become extremely fine, and with a sufficient excess of sensitizing reagent, the precipitation reaction with syphilitic sera will be completely inhibited.

Table VI illustrates the correction of an undersensitive antigen (Antigen IV) by the addition of sensitizing reagent.

### (c) *By addition to the antigen of sensitizing reagent plus dilution with cholesterolized antigen*

(1) Ten c c amounts of the undersensitive antigen are measured into two 25 c c Erlenmeyer flasks

(2) To one flask are added 0.05 c c sensitizing reagent (0.5 per cent) and 1.1 c c cholesterolized alcohol (10 per cent dilution) while to the other are added 0.1 c c sensitizing reagent (10 per cent) and 2.5 c c cholesterolized alcohol (20 per cent dilution)

(3) The two modified antigens are tested in the usual way, with standard antigen as a control. In case neither of the modified antigens corresponds in sensitiveness to the standard, other combinations of sensitizing reagent and cholesterolized antigen are employed.

Table VII illustrates the correction of an undersensitive antigen (Antigen V) by the combined use of sensitizing reagent and cholesterolized alcohol.

## 3 Summary of Methods of Antigen Correction

The following outline summarizes the methods of antigen correction.

<i>Types of Uncorrected Kahn Antigens</i>	<i>Methods Employed in Correction</i>
<i>Showing Variations in Sensitiveness</i>	

I Antigen Comparable in Sensitiveness to Standard Antigen	Correction unnecessary
-----------------------------------------------------------	------------------------

- |                                                  |                                                                                                                                                                      |
|--------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| II Antigen More Sensitive Than Standard Antigen  | (a) Dilution with cholesterolized alcohol<br>(b) Concentration of lipids<br>(c) Increasing amount of salt solution in antigen suspension beyond requirement of titer |
| III Antigen Less Sensitive Than Standard Antigen | (a) Dilution with cholesterolized alcohol<br>(b) Addition of sensitizing reagent<br>(c) Addition of sensitizing reagent plus dilution with cholesterolized alcohol   |

#### 4 Preferred Methods of Correction

(a) We believe it is desirable to employ as far as possible a standard titer for antigens. In this laboratory all antigens used in the 3 tube Kahn test during the past five years have been corrected to a standard titer of 1 + 11. We see no objection however to the employment of closely similar titers such as 1 + 12 or 1 + 13. Furthermore if an antigen can be corrected by a small increase (0.1 or 0.2 cc) in the salt solution used in preparing the suspension beyond the amount indicated by the titer this method should be found satisfactory. It should be emphasized that in the correction of antigen to standard sensitiveness, we have not found it necessary in most cases to retitrate the modified antigens before testing with sera.\*

(b) It is of interest to note that an antigen more sensitive than standard usually requires a comparatively large amount of salt solution in the titer. The preferred methods of correcting such antigens tend to reduce the amount of salt solution in the titer and at the same time decrease the sensitiveness of the antigen. One of these methods involves the dilution of the antigen with cholesterolized alcohol while the other involves the concentration of the antigen with its own lipids. The choice between these two methods is made as follows. Dilution of the more sensitive antigen with cholesterolized antigen is tried first. If the antigen can be corrected with an amount of cholesterolized alcohol not exceeding 20 per cent this is the method to be adopted. If the amount of alcohol required for correction exceeds 20 per cent the concentration method is preferred. Experience with highly sensitive antigens further indicates that those requiring excessive amounts of alcohol dilution for correction to standard sensitiveness, usually require but a small amount of lipid concentration for similar correction.

It occasionally happens that the entire amount of oversensitive antigen has been cholesterolized before standardization. When noncholesterolized antigen is not available it is obviously impossible to concentrate the antigen with its own lipids without thereby increasing the concentration of cholesterol be-

\* Correction with sensitizing agent may result in an increase in titer.

and its proper value of 0.6 per cent. In that case, one may add to the antigen in question the lipid residue from another noncholesterolized Kahn antigen.

(c) Turning to the methods of correction of antigens less sensitive than standard, the titer of the antigens gives an indication as to which method to employ. An undersensitive antigen having a titer which exceeds  $1 + 11$  ( $1 + 12$ ,  $1 + 13$ , etc.), indicating high concentration of lipids, is best corrected by dilution with cholesterolized alcohol. An undersensitive antigen which has a titer of  $1 + 1$ ,  $1 + 0.9$ ,  $0.8$  or  $0.7$ , indicating meager concentration of lipids, is most suitably corrected by adding sensitizing reagent and cholesterolized alcohol. Our experience indicates that antigens having titers of  $1 + 11$  are in practically all cases closely similar in sensitiveness to standard antigen. Should such antigens require correction, it will usually be found that dilution with cholesterolized alcohol is a suitable method. It should be added that the combined use of sensitizing reagent and cholesterolized alcohol in correcting undersensitive antigens has given better results in our hands than the use of sensitizing reagent alone.

#### REFERENCES

- 1 Kahn, R. L., Nagle, N., and Kendrick, P. L. Studies on Antigen for the Kahn Test I. Uniformity in Sensitiveness of Standard Antigen. *J Infect Dis* 41: 111-123, 1927.
- 2 Kahn, R. L. Serum Diagnosis of Syphilis by Precipitation. Williams & Wilkins Company, Baltimore, 1924.
- 3 Kahn, R. L. The Kahn Test, a Practical Guide. Williams & Wilkins Company, Baltimore, 1928.
- 4 Kahn, R. L., Lubin, G., and McDermott, E. Studies on Antigen for the Kahn Test II. A Sensitizing Reagent, *JOUR LAB & CLIN MED* 14: No 9, 876, June, 1929.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## ANEROBES A Simplified Method for the Cultivation of Anaerobes in Fluid Media Petroff S. A. Arch Path 5 834, 1928

Five or six holes 2 mm in diameter are made on the lower end of a tube that is from 10 to 12 cm long and 2 cm in diameter. The tube is well packed about one third with cotton. A rubber tubing with an inside diameter of 20 mm, an outside diameter of from 25 to 26 mm long is slipped over the absorption tube. The thickness of this tube will depend on the size of the mouth of the culture flask. The tube is then wrapped in paper in the usual way, sterilized at 15 pounds pressure and kept until needed. The broth media is inoculated as usual. The cotton plug of the flask is aseptically replaced with the absorption tube. Pyrogallol is placed in the tube on top of the cotton packing. Ten per cent sodium hydroxide is then slowly poured into the tube over the pyrogallol acid until the cotton packing is well dampened. An excess must be avoided. The absorption tube is then closed with a rubber stopper and the connections well sealed with paraffin.

## GRAM STAIN A Further Modification Kopeloff N and Cohen, P Stain Tech 3 No 2 64, 1928

Air dry film and fix with least amount of heat necessary

Flood with dye for five minutes. Previously mix 30 drops of a 1 per cent aqueous solution of crystal violet or methyl violet 6B with 8 drops of a 5 per cent solution of sodium bicarbonate. Allow the mixture to remain for five minutes or more.

Flush with iodine solution for two minutes. (Two grams iodine dissolved in 10 cc normal sodium hydroxide solution and 90 cc water added.)

Drain without blotting but do not allow film to dry.

Add a mixture of equal parts of acetone and alcohol drop by drop until the droppings are colorless (10 seconds or less).

Air dry slide

Counterstain for twenty seconds with 0.1 per cent aqueous solution of basic fuchsin.

Wash off excess stain by short exposure to tap water and air dry. If slide is not clear immersion in xylol is recommended.

## GLUCOSE SOLUTIONS The Prevention of Reactions Following Intravenous Injections of Glucose Solution Titus P and Dodds P Am J Obst & Gynec 14 No 2 181, 1927

The average dose for an adult is about one gram per kg of body weight. An average adult weighs between 50 and 75 kg so 75 gm is the initial dose subsequent doses to be 50 gm. There will be some spill through the kidneys but the appearance of sugar in the urine following such an injection is of no especial significance.

The glucose should be chemically pure. Several different preparations have been used and while there is little choice if chemically pure the authors have usually used Merck's c.p. anhydrous glucose.

The glucose should be dissolved in *freshly* double distilled uncontaminated water, and not in salt solution of sodium bicarbonate solution as has been variously recommended. In the preparation of a single dose 50 gm of the glucose is carefully weighed and then dissolved in 200 cc. of freshly distilled water, using only glassware which has been thoroughly washed in distilled water. Before preparing for sterilization the solution should be filtered



at least five or six times to remove even tiny particles of dust or cotton fibers. For the sake of simplicity in making any necessary calculations for dilutions it is convenient to have each flask contain 25 gm. of glucose in 100 cc. of water (25 per cent) of a "half dose."

The flasks of glucose solution after being properly stoppered with cotton plugs in gauze and the top sealed with lead foil, should be sterilized in a steam sterilizer for one half hour at 100° C. on three successive days. It may be prepared more quickly for emergencies by being sterilized in an autoclave at 15 pounds pressure for twenty minutes.

In either case sufficient space should be left between the level of the solution in the flask and the stopper so that the solution will not be forced by vacuum up against the stopper. This is also a precautionary measure against the possibility of splashing the solution against the stopper in any handling of the flasks.

Solutions showing caramelization or sediment after sterilization should be discarded.

Glucose solution kept in flasks stoppered with cotton, or cotton and gauze, and sealed with lead foil will show comparatively little change in the hydrogen ion content over a considerable period of time, even though buffer salts have not been added, so that solutions may be prepared in advance of the need for them.

Preferably solutions should not be kept longer than four weeks unless hermetically sealed, and the solution is probably safer when it is fresh. It is seldom, however, that a reaction can be traced to a solution that is not more than from two to four weeks old.

Ampules of glucose (dextrose) solution are now on the market and apparently have been carefully and properly prepared by their manufacturers. Many of these have been used with satisfactory results, and the only one open to particular comment is that of one firm to which cresol has been unnecessarily added as a preservative. This ampule contains only 10 gm. of glucose so that if enough ampules are used to obtain a therapeutic dose for anything other than insulin hypoglycemia, a reaction is a common aftermath on account of the total amount of cresol injected.

The injection should proceed slowly at the rate of 4 cc. per minute for a 25 per cent solution, and during this period the solution should be kept hot. The solution is ordinarily injected from a salvarsin tube, using a small caliber needle, and having the tube coiled in a basin of hot water to keep the temperature of the flowing fluid up to from 100° to 110° F.

Fresh rubber tubing should not be used until after it has been thoroughly washed in running water, then boiled in clean water (without sodium carbonate), again washed, and finally sterilized in the autoclave. Chemical contamination from new rubber tubing has been suggested as a cause of reactions following these injections.

The salvarsin tube and the connecting tubes, as well as the rubber tubing should be thoroughly cleansed and then sterilized in an autoclave, because when merely boiled, sediment from the water is often to be seen within the glass. Before beginning the injection the system should be rinsed out by running distilled water through the salvarsin tube, the rubber tubing, the connections, and the needle.

It is recommended that new rubber tubing should be treated before using as follows:

- (1) Soak in soap and water one hour.
- (2) Wash well with soap and water.
- (3) Wash in running water.
- (4) Soak for six hours in 4 per cent solution of sodium hydroxide.
- (5) Wash well in running water.
- (6) Wash well in distilled water.

#### COLLOIDAL GOLD A Method of Preparation, Leiboff, S. L. Arch. Dermat. & Syph. 17: 380, 1928

Clean the glassware by boiling for half an hour in a solution of Ivory soap, wash well with tap water and then soak for a few hours in warm chromic acid cleaning solution. This solution is prepared as follows:

Potassium dichromate	80 gm
Concentrated sulphuric acid	120 cc
Water	1000 cc

Wash well with tap water and lastly with distilled water and dry in a hot oven.

Prepare cresol phthalein indicator paper by immersing strips of fat free ashless filter paper into a 1 per cent alcoholic solution of cresol phthalein (1 gm of cresol phthalein in 100 cc of 90 per cent alcohol) and dry at room temperature.

In a large clean beaker place 1000 cc of ordinary distilled water and 10 cc of a 1 per cent solution of gold chloride in water. Add 1 gm of saccharose (cane sugar) and dissolve by mixing with a glass stirring rod. Add slowly drop by drop tenth normal sodium hydroxide mixing the contents all the while. From time to time take out one drop of the solution on the end of the stirring rod and place on a piece of the cresol phthalein indicator paper, when the end point is reached the spot of the paper touched by the solution turns pink. A good pink color should be obtained which should not fade in thirty seconds. It takes about 5 cc of the sodium hydroxide solution to produce the proper reaction. Place the beaker over a Bunsen flame and heat before the boiling point is reached a faint color will develop and when the solution begins to boil a beautiful clear salmon red gold solution will be obtained. Boil until no more color develops. It is better to boil it a little too long rather than not long enough. When cool titrate the solution as follows.

Into each of three test tubes place 5 cc of colloidal gold and add to the first tube 0.4 cc of a 1 per cent solution of sodium chloride to the second tube add 1.7 cc of 1 per cent solution of sodium chloride the third tube serves as a control. Let it stand for one hour at room temperature and follow one of the two following procedures.

If the first tube remains unchanged and the second tube is not completely reduced the solution is too alkaline and accordingly not sensitive enough. Set up seven tubes containing 5 cc of the colloidal gold each and titrate as shown in the table.

The tube containing the least amount of acid which remained unchanged is taken as correct and the amount of acid to be added is calculated from this.

If the first tube in the preliminary titration shows a slight reduction and the second tube is completely reduced the gold sol is slightly too acid and hence too sensitive. Set up seven tubes as indicated in the first procedure but add the corresponding amounts of hundredth normal sodium hydroxide instead of the acid.

TITRATION OF COLLOIDAL GOLD

TUBE	1	2	3	4	5	6	7 (control)
Number of cc of colloidal gold	5	5	5	5	5	5	5
Number of cc of hundredth normal hydrochloric acid	0.1	0.2	0.3	0.4	0.5	0.6	none
Number of cc of 1 per cent sodium chloride	0.4	0.4	0.4	0.4	0.4	0.4	0.4

A number of colloidal gold solutions prepared by this method were tested on known puritic syphilitic and normal spinal fluids and were checked by a colloidal gold sol prepared with triply distilled water by the formaldehyde process. Good agreements were obtained and in no instance was a reaction obtained with normal spinal fluids.

#### LIVER FUNCTION TESTS Practical Value of Liver Function Tests Piersol G M and Rothman M M J A M A 91 1768 1928

Of all the liver function tests thus far devised those of greatest clinical value and general usefulness are (1) the estimation of urobilinogen (2) the determination of serum bilirubin (either by the quantitative van den Bergh test or, better by the uterus index) and (3) the estimation of the degree of retention of the dye bromsulphthalein.

Urobilinogen is probably the most delicate single test for liver dysfunction. It is always increased even when the injury to the parenchyma is slight or when excessive blood destruction brings about an increase in bile pigment formation. In the authors experience urobilinogen is constantly increased to a noteworthy degree in portal cirrhosis. Slight increases in urobilinogen have been noted from time to time in a limited number of patients in whom liver disorder was suspected but not evident clinically.

In many conditions a persistent increase of urobilinogen was indicative of a residual hepatitis.

The presence of latent icterus, as revealed by serum bilirubin estimations, is of distinct importance both diagnostically and prognostically, because in this way even slight degrees of bile retention may be determined

The retention of bromsulphthalein is valuable confirmatory evidence of liver dysfunction. Such retention rarely occurs unless one or both of the liver function tests already mentioned are positive, with the exception of portal cirrhosis in which dye retention occurred in the absence of hyperbilirubinemia. In these authors' experience, 2 mg per kilogram of body weight of this dye is as accurate as the larger 5 mg dose.

**ACETONE BODIES** Citric Acid and Citrates as a Source of Error in the Van Slyke Method, Clarke, B E and Hausmann, G H Arch Path 6 No 5, 881, 1928

The authors demonstrate that sodium citrate used as an anticoagulant is a source of error in quantitative determination of acetone body content in blood by the method of Van Slyke. Citric acid administered by mouth does not have any effect on either the acetone body content or the power of the blood to combine with carbon dioxide. Citric acid and its salts are not a source of error in quantitative determination of acetone body content in urine by this method.

**Combined Nuclear and Differential Stain**, Brilmyer, G J Science 68 114, 1928

A new method for combining and manipulating Delafield's hematoxylin and Mallory's connective tissue stain is as follows:

(1) Stain in Delafield's hematoxylin, five minutes, (2) wash to remove excess of stain, (3) stain in 0.2 per cent aqueous solution of acid fuchsin one minute, (4) wash to remove excess of stain, (5) stain in the following solution two to three hours: Aniline blue (water solution) 0.5 gm, Orange G 20, and 1 per cent phosphomolybdic acid 100 cc, (6) wash to remove excess of stain, (7) pass successively and rapidly through 35, 75, and 95 per cent alcohol, (8) dehydrate in absolute alcohol thirty to sixty seconds (anhydrous acetone may be used in place of absolute alcohol), (9) clear in xylene, (10) mount with cover glass. With this procedure nuclei appear a rich red, epithelial cells pink, connective tissue blue and muscle red. Red blood corpuscles stain yellowish in veins, reddish in arteries. Colloid and mucus stain blue. Sections stained by this method have not faded in five years.

**MALARIA** Demonstration of Leishman Donovan Bodies and Malarial Parasites in Venous Blood, Mukherjee, H N Calcutta M J 22 No 9, 487, 1928

The fact that the corpuscles in blood from kala-azar and malaria cases sediment rapidly may be utilized for the detection of malarial parasites or Leishmanias. About 1 cc of citrated or oxalated blood taken from a vein is allowed to stand in long serologic pipette tubes for three to four hours. With a fine capillary pipette a little of the clear plasma just above the red blood cell deposit, together with about 1 mm of the topmost layer of red blood cells is removed and placed on a clean slide. A film is made and stained in the usual way. In sedimentation the uninfected red cells are at the bottom, those with malarial parasites next and the leucocytes in which Leishmania may occur above these.

**SPORES** Wright's as a Differential Spore Stain, Dutton, L O Stain Technic 3 No 4, 144, 1928

A heavy suspension of the organism to be stained is made in 0.4 cc of distilled water in a fairly large test tube (150 by 13 mm). To this suspension 0.1 cc of Wright's staining solution (50 to 100 mg to 60 cc of methyl alcohol) is added. The tube is then tightly stoppered to prevent evaporation of the alcohol and immersed in boiling water for about ten minutes. It is removed from the water bath and allowed to cool for about half a minute to prevent the contents from bubbling out when it is opened. Loopsful of the stained organism

are then spread on slides and dried. Examination shows the cytoplasm of the sporangium stained a reddish brown and the spore bodies a fairly intense blue.

If desired, a loopful of the organism may be mixed with an equal quantity of saturated aqueous solution of nigrosin as in the Donner technique and then spread on slides. The spores are then more plainly seen. Nigrosin weaker than saturated solution may be used with the Wright's stain, for it is a very powerful decolorizing agent for the red in the cytoplasm. As high a dilution as 1 to 100 renders the bacterial cell except the spore completely colorless.

**SCHISTOSOMIASIS. A Precipitin Test. Taliagerro W. H. Hoffman W. A. and Cook D. A. J. Prevent. Med. 2, No. 5, 395, 1928.**

1. Aqueous extracts made by extracting dried livers of *Planorbis guadeloupensis* containing larval stages of *Schistosoma mansoni* (1 cc. of extractive to 0.02 gm. powder) with the slightly alkaline solution of coca saline or phenol yield specific test antigens. Seventy-seven precipitin tests on the serums of twenty-eight persons with stools positive for *S. mansoni* gave 63 positive (20+++ 4++ and 10+) and 14 negative tests. 25 tests on five persons known to be negative for both *S. mansoni* and syphilis gave one positive (+) and 23 negative tests. 17 tests on four persons negative for *S. mansoni* but positive for syphilis gave 9 positive (2++ and 7+) and 8 negative. The active principle in these aqueous extracts is precipitated with the albumin fraction, i.e. it is not precipitated with half saturation with ammonium sulphate but is precipitated with entire saturation.

2. Attempts to prepare in vitro antigens by extracting the dried cercarial snail livers with N/20 or N/10 hydrochloric acid and N/20 or N/10 sodium hydroxide and adjusting the supernatant to pH 7.4 were all unsuccessful.

3. When antigens are prepared by a thorough extraction of the dried cercarial powder in a Soxhlet apparatus with either absolute alcohol or both followed by the extraction of the lipid free residue with coca's solution (containing 0.05 per cent  $\text{NaHCO}_3$ ) and the use of the clear supernatant (pH about 7.4 without further adjustment) the following advantages are found over the antigens noted in Section 1.

(a) Proteins and the reactive material go into solution much more rapidly so that antigens can be prepared in twenty minutes and all the antigens of each set are uniform.

(b) The reactions with schistosomal serums are much stronger. Thus the following results were obtained with antigens prepared from the same cercarial powder as in Number 1 but after lipid removal. 20 tests on eight known schistosomal cases were all positive (18+++ and 2++) whereas 20 tests on four nonschistosomal and nonsyphilitic serums were all negative.

(c) The cloudiness and spontaneous precipitation sometimes encountered with the antigens prepared as in Section 1 were completely eliminated by alcohol extraction and largely so by ether extraction.

(d) The pseudopositives in syphilitic serums were not eliminated by extraction with alcohol ether or both. Thus using the same lot of powder as in Section 1, antigens from the lipid free powder gave in 16 tests on four syphilitic cases not infected with *S. mansoni* 8 positive tests (3++ and 5+) and 8 negatives.

4. By centrifugation the larval schistosomes can be separated from the liver tissue in macerated fresh infected snail livers. This separation is so complete that the serums of two monkeys immunized against the normal snail liver gave a +++ reaction with antigens prepared from uninfected snail livers and whole infected snail livers but a negative reaction with antigen prepared from the concentrated larval schistosomes. Aqueous extracts prepared from the lipid free powder of these concentrated larvae are potent antigens and preliminary experiments suggest that they do not give pseudopositives with syphilitic serums.

5. Antigens prepared from lipid free powders of normal uninfected snail livers' give uniformly negative reactions with schistosomal serums.

**NEPHRITIS** The Andiewes Diazo Reaction In, Eastland, J S and Schmidt, E G  
Arch Int Med 43 No 4, 472, 1929

From a fairly extensive study of this reaction the authors conclude that

1 The Diazo reaction is a simple test for advanced renal damage which can be performed with the most meager laboratory equipment

2 A positive test was never found to accompany normal or low elimination of phenol sulphonephthalein

3 A positive test was always found to be associated with a marked retention of nitrogenous products

4 The test was found to be of considerable prognostic aid in the cases of advanced or extensive renal damage

**BERI BERI** The Etiology of, Matsumura, S et al, J A M A 92 No 16, 1325, 1929

The authors describe briefly a bacillus isolated from the feces in beriberi, not found in normal individuals, producing the disease in fowls, and agglutinated by the serum from cases of the disease

An organism resembling *B coli communior* was found, usually in relatively large numbers, among the intestinal types isolated from experimental animals and also from human cases. The microbe ferments saccharose readily, hence Endo plates containing this sugar in place of lactose lend themselves well for isolation

The organism, like *B coli*, is gram negative and motile and does not form spores. It has peritrichal flagella. It does not liquefy gelatin, it produces indol, coagulates milk and shows a rather distinctive colony on agar plates. It ferments the common hexoses, as well as maltose, lactose, saccharose, xylose, the hexatomic alcohols, mannitol and sorbitol, and glycerol, inulinose and arabinose, all with the production of gas and acid. Fermentation of dulcitol and arabin is irregular. Inositol, adonitol, erythritol, and a methyl glucoside are not fermented. The organism has been named *Bacillus beriberi*. The differentiation of *B beriberi* from *B coli communior* depends on the fact that the beriberi bacillus agglutinates and also gives a complement fixation reaction, *B coli communior* does not

**TUBAL PREGNANCY** The Pyramidone Test, Klein, S M Arch f Gynak 135 256, 1928

The author reports upon Jegeroff's modification in 68 cases

To a mixture of 3 cc of 5 per cent alcoholic pyramidone solution, 8 drops of 3 per cent hydrogen peroxide and 8 drops of 50 per cent acetic acid, add 1 drop of the serum to be tested. An amethyst blue color indicates the presence of hematin

A positive result was obtained in 90 per cent of cases of ectopic gestation. Positive results were also encountered in 2 cases of apoplexy ovarii and in 5 cases of intermenses

Klein believes the test of value in the diagnosis of tubal pregnancy

## REVIEWS

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Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building  
Richmond Va

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### *Serum Diagnosis by Complement Fixation\**

THE life of medical books is as a rule rather ephemeral for obvious reasons. This volume, however, it may be predicted will remain a standard text for years to come and in all probability will never be displaced as a fountainhead of information on this subject.

There are perhaps few laboratory procedures which have come into more common use than that commonly but in many ways improperly known as the Wassermann test. There are few procedures also which have been subjected to more misuse both in the manner in which it has been applied and the way in which its results have been interpreted and utilized as concerns the patient. For these reasons and many more which readily come to mind a clear cut and authoritative survey of the situation has long been demanded.

Few men have been more closely associated with the serologic study of disease than Dr Kolmer, none more responsible for the development of modern serologic technique and none better able to discuss it and its clinical applications.

That the laboratory worker, the syphilographer, the clinician and the pathologist may have at hand in one volume a clear, comprehensive and yet succinct survey of the methods utilized in the application of the complement fixation test in the field of serum diagnosis is the purpose of this book. That this purpose is excellently well fulfilled, the author's reputation would lead one to expect and the book itself fulfills all expectations.

Complement fixation tests in the minds of many suggest only their application to syphilis. That they have besides this a wide and varied use is less appreciated and that these applications are here detailed and discussed greatly adds to the usefulness of this volume.

This book is divided into four main sections.

In Part I (seven chapters, 87 pages) are discussed the principles of serum hemolysis and complement fixation. In Part II (2 chapters, 248 pages) the principles of complement fixation technique are detailed and in Part III (9 chapters, 80 pages) the minutia of the technique of complement fixation methods are fully covered.

The relation of Dr Kolmer to the development of complement fixation technique assures the reader of a complete, clear and authoritative presentation.

One is at loss whether to admire more the extent of the author's own investigations, the thoroughness with which every phase of the subject has been studied, the careful analysis of the literature or the way in which all these have been correlated and combined into a comprehensive whole.

These sections should be read by every worker who takes upon himself the performance of these tests, for therein every possible eventuality, every source of error, every puzzling phenomenon is noted and discussed to the advantage, not only of the laboratory worker who

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Serum Diagnosis by Complement Fixation With Special Reference To Syphilis. By  
John A. Kolmer, M.D., Professor of Pathology, Graduate School of Medicine, University of  
Pennsylvania. Cloth, Pp. 583, 65 Illustrations. Lea and Febiger, Philadelphia.

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NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

performs the test, but the clinician who utilizes its results, and who surely should be expected to understand the principles upon which it is based and the factors which may influence its results

In Part IV (15 chapters, 125 pages) are detailed the methods of obtaining various specimens and a most comprehensive yet succinct discussion of the chemical application of complement fixation tests

This book can be read with profit by all who are at all connected with the study of disease and its congeners. It will, however, be a difficult book to borrow as it is apt to be greatly in use

For the laboratory worker it furnishes a wealth of information concerning all the technical details involved in the conduct of complement fixation tests, not only in the study of syphilis, but in the study of varied diseases, and the identification of blood strains, meat and milk adulterations and so on, details of every phase from the preparation of the reagents to the conduct of the test

To the syphilographer it brings under one cover a comprehensive survey of all that is now known concerning the serologic study of syphilis

To the clinician it furnishes a mine of information of assistance in the understanding and interpretation of the serologic manifestations of syphilis

In the last analysis it suffices, perhaps, to say that the present volume is worthy to take its place beside the author's previous books, and there are few indeed who are unfamiliar with their worth

### *Laboratory Diagnosis and Experimental Methods in Tuberculosis\**

THE purpose of this book is to describe the more important methods of laboratory diagnosis, to consider the more significant experimental procedures in the study of tuberculosis, and to present such considerations as concisely as possible

The volume is divided into five principal parts. In Part One the general considerations of the body fluids and excreta in tuberculosis are discussed. Part Two is devoted to the bacteriologic diagnosis of tuberculosis, Part Three to the diagnostic use of tuberculin, Part Four to serologic diagnosis, and Part Five to methods used in the study of experimental tuberculosis

The author's aim, as stated in the preface, has been "to write a book which would be useful to practicing physicians, to public health officials, and laboratory workers in general, and to medical students"

In this he has been successful. The volume is concise but relatively comprehensive. While, of necessity, many methods of value have not been included, those which are discussed are mainly of demonstrated worth

The book can be read with profit and is a useful addition to the laboratory library and to the physician's armamentarium. Its study by the physician will undoubtedly decrease the too prevalent "inferiority complex" developed in the presence of the necessity for the diagnosis of tuberculosis in the doubtful case

### *Acute Infectious Diseases†*

THIS second edition of this well known work has been so extensively revised that, in many respects, it is almost a new work

The reputation of the authors is a sufficient guarantee of the comprehensive and authoritative character of the text

\*Laboratory Diagnosis and Experimental Methods in Tuberculosis. By H. S. Willis, Johns Hopkins Hospital, with a chapter on Tuberculo-Complement Fixation by J. S. Woolley, Loomis Sanitarium, and an Introduction by Allen K. Krause. Cloth. Pp. 330. 25 Illustrations. C. C. Thomas, Springfield, Ill.

†Acute Infectious Diseases. By J. F. Schramburg, Professor of Dermatology and Syphilology, Graduate School, University of Pennsylvania, and J. A. Kolmer, Professor of Bacteriology and Pathology, Graduate School, University of Pennsylvania. Second Edition. Thoroughly Revised. Cloth. 161 engravings, 27 full-page plates. Lea and Febiger, Philadelphia.

As stated in the preface "It is a sad commentary upon the intelligence and judicial mindedness of certain elements in the community that it is still necessary to stress the evidence in favor of vaccination."

The present unenviable position of the United States in the World list of smallpox incidence is sufficient evidence that the lessons of the past are not being remembered. It is exceedingly timely therefore that a comprehensive and accurate resumé of the vaccination question is available and even the physician will find much of interest in this section of the book.

In the section concerned with diphtheria prevention a subject now very much to the fore in the public mind will be found a very excellent discussion of toxin antitoxin and toxoid immunization.

The authors believe that toxoid immunization has a promising future if present observations are supported by future experience.

The entire volume bears the stamp not only of the extensive experience of the authors but of a careful and comprehensive review of the literature. It may be accepted as presenting in a most excellent manner the last word to date of modern medicine on the subject of acute infectious. It is a book which the physician can ill do without.

### Serodiagnosis of Syphilis\*

IN THIS small paper covered volume is presented in succinct fashion the methods used by the author for the conduct of complement fixation and flocculation reactions in the serologic study of syphilis.

### Preventive Medicine†

THE present edition of this useful book has been comprehensively revised to include the advances in this important field and may be recommended as authoritative and reliable.

### A Laboratory Manual of Physiological Chemistry‡

A TEACHING manual intended for use in medical, dental, or veterinary schools and eminently suitable for the purpose. The book is interlarded for the convenience of the student.

### Recent Advances in Bacteriology§

IT WOULD be difficult to select from those concerned with the study of disease any to whom this book would not be useful: the student, the public health worker, the physician, the pathologist, all will find it of interest and value.

The author in his preface says: "The endeavor has been made to take a broad view of many subjects and in keeping the balance between extreme technicality and what is already common knowledge to present a readable exposition of recent work which the general medical reader, not himself especially versed in bacteriology, may appreciate." At

\**Précis de Technique du Serodagnostic de la Syphilis*. By R. Demanche. Paper 132 pages. Gaston Doin et Cie, Paris.

†*Preventive Medicine*. By W. F. Boyd, M.D., Member of Full Staff, International Health Division of the Rockefeller Foundation. Cloth, 475 pages. Third edition revised. W. B. Saunders Co., Philadelphia, Pa.

‡*A Laboratory Manual of Physiological Chemistry*. By D. Wright Wilson, Professor of Physiological Chemistry, Univ. of Penna. Cloth, 300 pages. Williams and Wilkins Co., Baltimore, Md.

§*Recent Advances in Bacteriology and the Study of Infection*. By J. H. Dible, Professor of Bacteriology and Pathology in the Welsh National Medical School. Paper 363 pages. Illustrations. P. Blakiston's Son & Co., Philadelphia.



the same time the author seeks to indicate to those who do possess some knowledge of bacteriology and the infectious, what is being done in spheres outside their own "

The scope of the volume is a sufficient evidence that these aims have been very well accomplished

There are chapters on the streptococcus problem, bacterial variation, the bacteriophage, Calmette and B C G, ultra microscopic viruses, diseases associated with rickettsia bodies, measles, recent work upon pneumococci, upon spirochetal infections, on diphtheriae and anaerobes, and local immunity

All these are thoroughly and carefully renewed and in an interesting and readable manner The author shows not only an intelligent mastery of his subject but demonstrates an ability to make it clear to others and to do this in an interesting way

He has very wisely restricted the bibliography to the papers referred to in the text which are, also very wisely, those referring to more recent work

This volume can be very highly recommended

The format and typography are commendable

### *A Handbook of Clinical Chemical Pathology\**

THE clinician desiring to utilize to the fullest advantage the resources of modern medicine soon realizes the difficulty of preparing textbooks or systems which are in all respects consistently in step with rapidly changing subjects such as the application of chemistry to the study of disease

There is a place, therefore, for a succinct yet accurate statement as to the best utilization of such methods and their clinical interpretation

This is the purpose of this small volume, the first the reviewer has seen from abroad although similar American texts have appeared before

This little book should be decidedly useful to the practicing physician and lead to a better utilization of the chemical aids now available to the management of disease

### *Thrombo-Angitis Obliterans†*

THIS very timely little monograph from the Mayo Clinic is based upon a careful and systematic study of more than 300 cases, fifty amputated specimens being studied pathologically

This peculiar disease, peculiar in its age and sex frequency and racial predilection, has been the subject of increasing interest in recent years and the present volume presents, not only a summation of the important recent contributions concerning it, but a very excellent intensive study demonstrating many facts of practical interest and suggesting inferences of marked importance

During the years 1922 to 1927 inclusive the ratio of patients with this disease to all males registering at the Mayo Clinic has been 1 400

The authors do not regard tobacco as a primary etiologic agent but believe it may be a contributing factor It is probable, they say, that the racial element depends largely on the clientele and geographic distribution of the physicians who are interested in this disease and that the predominance of the disease in males is related to differences in occupation

The possible infectious nature of the disease has been the subject of much study The authors hold that, while proof is lacking, there is much evidence in favor of an infectious or bacterial toxic agent as the etiologic factor, although, of course, there are many con-

\*A Handbook of Clinical Chemical Pathology By F S Fowweather Lecturer in Chemical Pathology University of Leeds Cloth Pp 216 18 illustrations P Blakiston's Son & Co Philadelphia

†Thrombo-Angitis Obliterans Clinical Physiology and Pathologic Studies By George E Brown M.D. and Edgar V Allen M.D. Division of Medicine Mayo Clinic Collaborating in Pathology with Howard R Mahorner M.D. Fellow in Surgery The Mayo Foundation 219 pages 62 illustrations Cloth W B Saunders Co Philadelphia and London 1928

tributing elements such as the exposure, occupation, etc. They believe that an intensive bacteriologic study is in order.

The results of their pathologic study suggest that thromboangitis obliterans is fundamentally a chronic inflammatory condition of the vessels accompanied by proliferation of the intima and resulting in thrombosis with organization and canalization of the clot fibrosis of the intima, and an attempt on the part of the vasa vasorum and other collateral channels to establish a collateral circulation. The nerves are involved by virtue of their relationship to the vessels and by ischemia in the distal portions.

In the chapters on the clinical course, clinical types, analysis of symptoms, diagnosis and treatment there is a wealth of practical material very clearly discussed.

The studies of recent years have very materially modified the previous dictum of "high and early amputation" and demonstrated that

- 1 In selected cases low amputation is successful
- 2 In many cases severe pain is relieved by medical measures
- 3 In many cases without trophic changes gangrene can be prevented
- 4 Small trophic ulcers can be healed frequently
- 5 Lumbar ganglionectomy is occasionally of value
- 6 Many patients go through the course of the disease without trophic changes or gangrene and eventually reach a stage of adequate compensation.

Although therefore a high degree of individualization is necessary the authors believe that the type of treatment can be determined by the clinical type of the case when first seen.

The chapters on treatment, prophylaxis and prognosis well repay careful reading.

A special chapter is given to special methods of investigation.

The typography of the book is excellent.

This volume is a very valuable contribution to the subject and should be in the hands of every physician, most especially those particularly interested in this condition.

### *Morphologic Variation and the Rate of Growth of Bacteria\**

THIS is Volume I of a projected series of Microbiology Monographs designed to cover the field of general agricultural and industrial microbiology.

Microbiology is assuming such broad outlines that the only way in which the various phases of this comprehensive subject can ever be covered is by monographic presentation. This volume therefore will be welcomed as a thorough, authoritative, clearly written presentation of a rather complicated phase of a far from simple subject.

Fortunately for the average reader Dr. Henrici admits in his preface that he is no mathematician. "The expert in biometrics," he says, "will therefore find here no such carefully fitted curves and elaborate formulae as delight his heart, but the ordinary person may follow my argument with only a very elementary knowledge of statistics." The subject being one in which the "ordinary person" interested in microbiology must be cognizant of this is indeed a consummation devoutly to be wished for!

Recent developments in bacteriology have given a death blow to the old monomorphic concept which so long dominated the field. The evidence presented by Dr. Henrici makes plain to the average reader just what has been done and what is being done in the study of morphologic bacterial variation of senescent forms and of cytomorphosis.

There is a chapter on technique for those desirous of pursuing similar studies, an appendix of tables and a complete bibliography of the titles referred to in the text.

The author is to be congratulated upon a valuable contribution and the publishers upon an excellent example of craftsmanship.

*Morphologic Variation and the Rate of Growth of Bacteria.* By A. T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Cloth. Pp. 194. 36 figures, 27 tables. C. C. Thomas, Baltimore, Md.

### *The Fuel of Life\**

IN THIS volume we presented four lectures delivered under the Louis Clark Vanuxem Foundation of Princeton University

Dr MacLeod here presents in a clear and masterly manner what is known at present of the preparation of food materials for combustion in the animal organism, especially as concerns the question as to whether fats as well as proteins form carbohydrate before being used as fuel

Much of the data presented is based, very naturally, on investigations conducted under Dr MacLeod's supervision

His own hypothesis, which these lectures in large measure support, is centered upon the work of Hill and Meyerhof, that the energy of muscular contraction is based largely, if not entirely upon the combustion of carbohydrate According to his theory neither fat nor protein is burned by the muscles until it has been converted into carbohydrate or some related substance, mainly by the liver

To present the evidence accumulated in support of this theory is the purpose of the lectures

The volume is of great interest to physicians, physiologists and all directly or indirectly concerned with human metabolism

It can be read not only with profit but with interest A bibliography and a very complete index are included

### *Organic Laboratory Methods†*

IT IS difficult to conceive a chemical laboratory in which this book will not be received with enthusiasm

The analytical chemist will find in it the solution of many difficulties As a reference source it should be invaluable, especially as it appears to be the only book of its kind

It may be purchased with entire confidence that it will be useful

### *Urinary Analysis and Diagnosis‡*

THE outstanding feature of this book is the exhaustive and detailed study of the formed elements in urine sediments The author is firmly convinced, and makes an excellent argument for his case, that much valuable information is lost by neglect of careful study of the epithelium in urine

The chemical examination of urine is handled in orthodox style though no mention is made of some of the newer and valuable methods such as Exton's method for quantitative albumin determination, for example That portion of the book concerned with microscopic examination is the best and, if only for this portion, the book is a good reference work

### *Neoplastic Diseases§*

DR EWING's masterly treatise on tumors needs no introduction to the medical world for it is doubtful if there is a library of any worth in which it cannot be found showing the honorable scars of frequent use

\*The Fuel of Life By J J R MacLeod Professor of Physiology University of Toronto 147 pages Cloth Princeton University Press Princeton

†Organic Laboratory Methods Professor Lassar-Cohn Authorized translation by R C Oesper Ph D Assistant Professor of Analytical Chemistry Univ of Cincinnati Cloth 469 pages 186 figures Williams and Wilkins Co Baltimore Md

‡Urinary Analysis and Diagnosis By L Heltzmann MD Fifth Revised Edition 131 illustrations 366 pages W Wood & Co New York

§Neoplastic Diseases By James Ewing MD ScD Professor of Pathology at Cornell University Medical College New York City Third Edition Revised and Enlarged Octavo of 1127 pages with 546 illustrations Cloth 1928 Philadelphia and London W B Saunders Co

This the third edition has been thoroughly and extensively revised every chapter showing numerous additions

The chapter on Bonus is practically new having been entirely rewritten The subjects of mammary cancer and brain tumors are also in large part rewritten

None who have read the previous editions will want to be without the present edition of this masterly work

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### *The Kahn Test<sup>1</sup>*

THIS volume presents with a wealth of detail the various minutia applying to the technique of the Kahn test and is intended primarily for use in the laboratory

It will be found of great value to all who are interested in this method and its study will without doubt conduce to its performance in a uniform manner

This reviewer has always regarded the Kahn test as a valuable *adjunct* to the serologic study of syphilis and has always opposed the clamor for its *exclusive* use especially when urged on the ground of simplicity of technique He is glad therefore to welcome this book which so clearly sets forth the inherent complexities of the reaction and the necessity therefore for its performance by trained workers

An extended bibliography is appended of papers concerning the Kahn test None of these, however are discussed in the text

This book should be in the hands of every worker who essays the Kahn test

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## EDITORIALS

### Our Carbon Monoxide Environment

ALTHOUGH present-day conveniences, such as the gas range, the central heating plant, and the automobile have increased the possibility of toxic carbon monoxide exposure, this poison like most other things of which we today take cognizance was a feature of ancient antiquity Shumway tells us that Aristotle, three hundred years before Christ, wrote that "men suffer from heaviness of the head and often die from coal gas" Valerius Maximus wrote that "Hannibal forced the inhabitants of Nuceria, each with two suits to leave the city, whereupon he prepared baths and caused the people to be suffocated in them by steam and smoke" Thus it was that two hundred years before Christ water gas was an accepted method of execution

From this we can readily understand that no definite date can be fixed as the time at which carbon monoxide came to be seriously considered as a

cause of disease. Attention was however focused on it in the last decade of the last century by the work of Haldane who was called upon to make a study of the lethal gases in the London tubes in which the trains at that time were drawn by coal burning locomotives. Haldane concluded after an exhaustive study that the concentration of carbon monoxide in the tunnels should not be allowed to exceed one part per ten thousand.

In recent years Henderson and Haggard have made comparable studies in connection with the vehicular tunnel beneath the Hudson River. They concluded that the maximal allowable carbon monoxide concentration might be four parts per ten thousand. In the course of their investigation they formulated a general rule of toxicity which is easily applicable to all such studies and which at the same time reconciles their allowable content with the lower content recommended by Haldane in England.

They believe that when the duration of exposure expressed in hours multiplied by the carbon monoxide concentration expressed in parts per ten thousand gives a figure not over three no symptoms of intoxication will result. When the exposure time multiplied by the concentration gives a figure of six symptoms become barely recognizable. When exposure times concentration equals nine, symptoms such as palpitation, headache, vertigo and sometimes nausea ensue and when the product equals fifteen the result may be serious.

In the London tube the train crews were to be exposed for several hours at a time. Six hours exposure according to Henderson and Haggard's table to a concentration of one part in ten thousand would give barely recognizable symptoms and it is therefore easy to understand why the concentration must be kept below this figure. In the Holland tube beneath the Hudson, four parts per ten thousand are allowed on the calculation that a maximum of one hour might be required to make the traverse of the tunnel. The investigators further stipulated that the guards at their posts in the tunnel must have specially purified air of a lower concentration.

When we observe that various investigators have agreed that the air in congested streets in our large cities at the peaks of automobile traffic often equals one part per ten thousand and not infrequently even two parts we must realize that there is actually a carbon monoxide problem. In congested downtown districts we are sometimes actually breathing air that is more vitiated with carbon monoxide than is allowed in the underground tunnels of London.

As one would expect those exposed for prolonged periods in this vitiated atmosphere actually do experience symptoms therefrom. A careful study has been made by Wilson, Gates, Owen and Dawson of the degree of carbon monoxide absorption among Philadelphia traffic officers. Where the exposure was for relatively long intervals in the congested traffic district they found as high as 30 per cent saturation of the hemoglobin in the blood with carbon monoxide and these officers experienced definite symptoms such as rapid pulse and respiration, palpitation and after slight exercise dizziness and amblyopia. Headache and nausea were not infrequent manifestations.

Connolly in investigating the carbon monoxide hazard in city streets found that on a double decker drive the vitiation of the air on the lower

deck where breezes could not carry away the gases satisfactorily, remained high enough to be a source of danger to any who might be exposed for a sufficiently long interval. And immediately adjoining this lower deck were windows of tenements in which the occupants spent a good portion of the day. These people certainly are living in a carbon monoxide environment.

The air vitiation in garages often becomes too great for health, and there are records of many cases of poisoning therefrom. Bloomfield and Isbell in a study of a relatively large number of garages through the country found an average carbon monoxide concentration of from 1 to 16 parts per ten thousand. At times the content was as high as 89 parts per ten thousand. The maximum average concentration observed for a period of one hour was 43 parts per ten thousand.

The New York division of Industrial Hygiene made, in 1923, a survey of 157 garages, service stations and repair shops. In the workers in these places 69.5 per cent showed some carbon monoxide in the blood, 77.5 per cent of the garages and other places showed appreciable amounts of carbon monoxide in the air.

A man's blood may become over 20 per cent saturated with monoxide while he is working for one hour in a garage containing four parts per cent thousand concentration.

The exhaust gas from automobiles varies in its carbon monoxide content depending upon carburetor adjustment but it usually contains from four to six or occasionally 10 per cent carbon monoxide. This is rapidly diluted so that a man standing behind a car which is not in motion but in which the engine is going will be in an atmosphere of about four parts per ten thousand. Thus if you are held up in traffic behind an idling engine you are in a decidedly vitiated atmosphere.

We should further bear in mind that physical exertion increases the oxygen demand and therefore will result in symptoms of poisoning earlier than where the victim is at rest. Henderson and Haggard's tables of exposure times concentration mentioned above are for individuals at rest and the effect is doubled or even tripled by exercise. In other words for a person at rest an exposure of three hours in a concentration of three parts per ten thousand will give rise to symptoms, while with an individual at work an exposure of but one hour to the same concentration will theoretically give rise to as severe symptoms.

Acute carbon monoxide poisoning, more often than not, fatal, is well known and incidences thereof are described frequently both in the medical and lay presses. Where death has followed illuminating gas poisoning or the running of a motor in a closed garage the situation is usually obvious at once. The rapidity with which such poisoning may occur is illustrated in the work of Henderson who says that in a closed garage ten feet broad, ten feet high and twenty feet long containing two thousand cubic feet capacity, a car delivering one cubic foot of carbon monoxide per minute will vitiate the atmosphere in one minute, and in five minutes the concentration will reach twenty parts per ten thousand. Beck states that in such a garage the danger point is reached within three minutes and asphyxia may occur in

five minutes. Illuminating gas which often contains as high as 30 per cent carbon monoxide exerts its deadly effect as rapidly. In an atmosphere containing 1 per cent carbon monoxide the victim's hemoglobin will become 50 per cent saturated with carbon monoxide within fifteen minutes, and in twenty minutes coma will ensue.

Carbon monoxide has from 220 to 250 times the affinity for hemoglobin that oxygen possesses. Symptoms appear when the hemoglobin is from 20 to 30 per cent saturated. When the saturation reaches 50 to 55 per cent walking is difficult and the victim becomes unconscious at 60 per cent saturation. Dogs die in a carbon monoxide hemoglobin saturation of 84 per cent. In illuminating gas they die when the carbon monoxide saturation is 72 per cent. Therefore there is something in illuminating gas besides the pure carbon monoxide that also exerts a toxic action.

But we have said that the acute fatal case is obvious. It is the chronic case, the case of the individual exposed to low concentrations over long periods that is worthy of special attention for there can be no doubt that hundreds of such cases are overlooked daily in the routine work of the physician. One need but be on the alert for such cases to find them. The stenographer working in a closed room heated by a portable gas heater or at an open window in an air shaft into which a neighboring chimney is belching smoke, or on the ground floor down town where motor traffic is heavy may complain to you of headache and you may inquire into the efficiency of the office illumination or you may send her to have glasses fitted or you may search for focal infection and chronic toxemic absorption from the colon but the cause of the headache may be none other than carbon monoxide poisoning in its chronic form.

The textbooks dwell at no great length upon this disease nor is much time allotted to it during undergraduate instruction. Were it otherwise there would probably be many more descriptions of carbon monoxide poisoning in the literature.

The pathology is simple. It has apparently been demonstrated that carbon monoxide is not in and of itself a direct tissue poison. Its only activity is a combining with the hemoglobin to the exclusion of oxygen and the resulting pathologic changes are those of anoxemia. Henderson was able to grow embryonic chick en brains in chick plasma in a concentration of 21 per cent oxygen and 79 per cent carbon monoxide. Hemoglobin does not enter into this procedure and the available oxygen is normal. The 79 per cent carbon monoxide behaved as an inert gas just as nitrogen does in our own respiratory air. If an animal breathes oxygen under two atmospheres pressure thus rendering the oxygen content of the blood independent of hemoglobin the addition of carbon monoxide in any amount produces no symptoms.

The pathologic physiology being an anoxemia we may readily understand how practically any tissue of the body may undergo pathologic change. The nervous system being most susceptible to anoxemia usually shows the more extensive changes but in fatal cases, especially, other organs and tissues may suffer. Briefly the changes in the nervous system consist in perivascular



hemorrhages followed by softening. Any part of the nervous system may be so affected. The lenticular nucleus appears to be especially susceptible.

Alpers describes a nonfatal case of poisoning in which the nervous manifestations indicated damage to the lenticular nucleus, the pyramidal tract, the bulbar nuclei and the peripheral nerves in the form of a peripheral neuritis. R. M. Stewart describes a fatal case with bilateral softening of the corpus striatum, softening and hemorrhages of the deeper layers of the cortex and cerebellum, but with the chief damage in the corpus striatum. Ruge describes twelve cases of carbon monoxide poisoning with softening and changes in the vessels of the brain, especially in the lenticular nucleus. He observes in moderately severe experimental poisoning, fatty degeneration in the ganglion cells within twenty-four hours of exposure, with small hemorrhages in the perivascular spaces. After two days, softening of the lenticular nucleus appears but this is not well marked until about the fourth or fifth day.

There may be scattered small hemorrhages and intense hyperemia of all body organs. There may be a great variety of eye lesions such as scotomas, color blindness, extraocular palsy, engorgement of the retinal vessels, impairment of the pupillary reflexes, irregularity of the pupils, nystagmus, edema of the optic discs followed by secondary optic atrophy and blindness, retinal hemorrhages or even complete ophthalmoplegia. Blindness is rare but when it does occur it is usually due either to an optic neuritis or to central changes, presumably softening in the region of the visual fields.

It has been said that chronic carbon monoxide poisoning does not occur unless the fixed carbon monoxide in the blood amounts to at least 5 per cent of the hemoglobin saturation. Fortunately methods are available by which the carbon monoxide saturation may be determined down to 1 per cent.

Early symptoms of monoxide poisoning are "toxic." They consist of yawning, sleepiness, weariness, constriction across the forehead, frontal headache, at first intermittent but later continuous and more severe. This headache is later replaced by the typical monoxide headache located at the base of the skull, in the occipital region, extremely severe, causing the sufferer to hold his head back as far as possible. This typical headache has been attributed to increased intracranial pressure.

Then come dizziness and nausea, rapid heart and respiration, cardiac irregularity and finally stupor. Vomiting sometimes occurs. After recovery the headache, weakness and often loss of memory persist for several days.

The symptoms of chronic monoxide poisoning are rather more insidious. Shumway describes them as headache, palpitation, anorexia, vertigo, anemia, eyestrain, general fatigue, lack of concentration, neuralgic pains, congested nasal and ocular mucous membrane, dry throat, irritation and watering of the eyes, lack of decision, restlessness, irritability, insomnia, and sometimes visual and auditory hallucinations.

These are the usual symptoms of many chronic intoxications and this is the more reason why the physician should bear in mind the present-day frequent sources of chronic carbon monoxide poisoning. As Lewin of Berlin states, there is no poison which is known to give so wide a variety of patho-

logic changes as carbon monoxide. The same could almost be said for its symptoms.

Beck has made an especially comprehensive study of the symptomatology of chronic monoxide poisoning and its manifestations are surely protean. At first there is a compensatory polycythemia with an increase both in the red count and in the hemoglobin concentration. Later this gives place to an anemia which may often closely resemble pernicious anemia. Indeed it may have many of the characteristics of pernicious anemia including the atrophic glossitis, the gastric achylia, asthma, high color index and even the paresthesias which in pernicious anemia are customarily due to a postolateral sclerosis. Beck further remarks that in chronic monoxide poisoning the glossitis may be present even without the anemia and its observance should remind one of the possibility of this form of poisoning. According to this author abdominal symptoms are not uncommon and are due to increased tonus of the smooth muscle system. The symptoms may even be so acute and severe as to suggest perforation of a gastric ulcer or renal colic. He describes cases clinically resembling hyperthyroidism with exophthalmos, sweating, etc. due to the action of the poison on the autonomic nervous system but in which the pulse and basal metabolic rate remain normal.

Usually psychasthenic and neurasthenic symptoms are prominent. Beck emphasizes the diagnostic significance of a symptomatology usually attributed to anemia in an individual whose blood examination actually shows a polycythemia.

The earliest and most constant eye-ground changes consist in congestion in the retinal vessels and hyperemia of the optic discs. These are worth looking for in a suspected case.

We should bear in mind that children are much more susceptible to carbon monoxide poisoning than adults and that a child may even be fatally poisoned in a room in which an adult companion experiences little or no symptoms. Abt reports the case of a child who although not fatally poisoned was blinded permanently from exposure to illuminating gas while playing in the same room with his mother who at no time was conscious of toxic symptoms.

This phase of the situation is especially important with infants who are unable to describe their symptoms or to ask for help. Stevens describes the evidences of chronic monoxide poisoning as failure to gain in weight, attacks of vomiting, restlessness, constant crying, anorexia and night terrors. It is only in the advanced cases that we observe the telltale pink flush of the skin, the slow pulse and the subnormal temperature. Here again the symptoms are symptoms that might be attributable to any of many causes and this is so much the more reason for bearing the possibility of monoxide poisoning in mind. Proof that the symptoms are due to this cause may be obtained by examination of the blood or by changing the environment after which the infant commences to flourish.

Fortunately for the acute case the poison is eliminated relatively rapidly, the major portion being removed in from three to five hours but minute amounts may persist in the blood for two weeks or longer, and where this is

cumulative and the exposure to small amounts continues, chronic poisoning may supervene

Fortunately the means are available for recognizing carbon monoxide either in the atmosphere or in the blood. Air samples may be obtained over varying intervals by drawing air into properly arranged tanks, or instantaneous specimens may be obtained by the so-called *grab* method. A mason jar filled with water suddenly inverted allows the replacement of the water with air from the locality to be tested. The cap is then quickly applied. Another grab method consists in inflating the bladder of a basket ball with an air pump. In this case the determination must be made relatively soon thereafter to prevent the diffusion of gases through the rubber.

Probably the best method for air analysis in detecting carbon monoxide is the iodine pentoxide method. A quick qualitative method consists in exposing a saturated solution of lime water to the air. Carbon monoxide causes rapid clouding with precipitated calcium carbonate.

For examination of the blood the Van Slyke and Salvesen method is accurate down to 1 per cent. A rapid qualitative method which will establish the presence of 10 per cent or over of monoxide hemoglobin saturation consists in taking three or four drops of blood in five c.c. of distilled water and then adding a drop or two of 5 per cent sodium hydroxide. Normal blood turns a dirty green with this procedure while monoxide blood remains unchanged in color, preserving its original pink, or if more pronounced, cherry red color.

#### REFERENCES

- Abt, I. A. *Med Clinics of North America* 5 1645, May, 1922  
 Alpers, B. J. *Am J Med Sc* 170 390, September, 1925  
 Beck, H. G. *Annals Clin Med* 5 1088, June, 1927  
 Bloomfield, J. J., and Isabell, H. S. *Public Health Reports* 43 750, March 30, 1928  
 Connolly, J. T., et al. *Am J Pub Health* 18 1375, November, 1928  
 Henderson, Y. *Boston Med & Surg J* 187 180, August 3, 1922  
 Henderson, Y., and Haggard, H. W. *J A M A* 81 385, August 4, 1923  
 Ruge, H. *Arch f Psychiat* 64 150, 1921 to 1922  
 Shumway, E. A. *M J & Record* 121 657, June 3, 1925  
 Stevens, A. M. *J A M A* 86 1201, April 17, 1926  
 Stewart, R. M. *J Neurol & Psychopath* 1 105, 1920 to 1921  
 Van Slyke, D. D., and Salvesen, H. A. *J Biol Chem* 40 103, November, 1919  
 Wilson, E. D., Gates, I., Owen, H. R., and Dawson, W. T. *J A M A* 87 319, July 31, 1926

—W T V

## The Oral Administration of Typhoid Vaccine

THE oral administration of bacterial vaccines was proposed as long ago as 1904 and ever since has been the subject of sporadic investigation with rather indefinite results

Following some rather *promising results in animals* Besredka<sup>1</sup> in 1922 during an epidemic immunized 268 students by the oral administration of typhoid vaccine, a similar group of 253 being vaccinated subcutaneously. In the first group five new cases developed after eleven days while ten new cases developed after twenty days in the second group

Results similar in character are reported by others notably Gauthier who reports 4000 cases

Opposed to this is the report of Achard and Blach<sup>2</sup> who failed to find either agglutinins or complement fixing bodies after oral vaccination

Because of the paucity of experimental evidence the question has again been studied by Haffstadt and Thompson<sup>4</sup>

A group of 93 university students from nineteen to forty years of age furnished the experimental material 5 students being vaccinated subcutaneously as controls

Of the experimental group 55 received 1 c c of triple typhoid vaccine in a glass of water one half hour before breakfast on three consecutive days and 38 received two capsules each containing 15 grains of alcoholic extract of ox bile with the first of the three doses of vaccine

The first group were tested after five and nine months and the second after five months for the presence of agglutinins precipitins and complement fixing bodies

Agglutinins were tested for by the macroscopic method a total of 30,000 agglutination tests being made during the experiment with the results summarized below

The systemic reaction was much less after oral than subcutaneous vaccination. Of the entire group of 93 88.5 per cent showed agglutinins for typhoid and a lesser number for the paratyphoids the agglutinin titer being higher in the bile prepared group the bile appearing to hasten slightly agglutinin production. The majority of cases showed a latent period of three to four weeks. The highest agglutinin titer for typhoid was 600, for paratyphoid 100, the maximum titer being reached by the time of the last subcutaneous inoculation and in the fourth and fifth week after oral ingestion of the vaccine. The disappearance of the agglutinins was too varied to warrant any generalization

From this portion of their study the authors concluded that

- 1 Agglutinins can be produced by the oral administration of triple typhoid vaccine

- 2 There is no delay in the appearance of agglutinins thus produced

- 3 Bile seems to aid agglutinin production

4 Previous treatment or history of typhoid fever affects only the agglutinin titer of the individual

5 Immunity may be demonstrated nine months after treatment

In a continuation of their study Haffstadt and Thompson investigated the incidence and degree of production of precipitins and complement-fixing body production in the group (39) subjected to oral vaccination

Precipitins appeared in 66.6 per cent and complement-fixing bodies in 60 per cent. The administration of bile was without apparent influence on the production of these immune bodies. The latent period for complement-fixing bodies is four to five weeks, precipitins appearing somewhat earlier. The disappearance of these antibodies is irregular and their duration variable.

There seems to be little relation between the presence of the various antibodies, but few individuals showing all three, and neither precipitins nor complement-fixing bodies being consistently found. There was, however, an apparent relation between the appearance and disappearance of complement-fixing bodies and agglutinins.

A still further study showed that entirely similar immunologic results were obtainable after the oral administration of the vaccine in capsules prepared after the formula following:

A suspension containing four billion typhoid organisms (Rowling's strain), 28 million para A and 28 million para B per centimeter was titrated with a matrix of starch until the moisture was absorbed. After adding an alcoholic extract of bile it was dispensed in 00 capsules each containing 0.25 cc of the original suspension and 1.5 grains of ox bile. One capsule was given one-half hour before breakfast on three successive mornings.

The experimental evidence thus adduced entirely corroborates the clinical evidence heretofore reported and demonstrates a scientific basis for oral typhoid vaccination.

#### REFERENCES

- 1 Besredka, A. Local Immunization, p. 133, Williams and Wilkins, 1927
- 2 Gauthier, A. Vaccination by Mouth Against Typhoid, *Bull de l'Acad de med* 91: 486, 1924
- 3 Achard, Kl, and Blach, P. Typhoid Vaccination by Mouth, *Bull de l'Acad de Med* 91: 531, 1924
- 4 Haffstadt, R. E., and Thompson, R. L. Immunological Studies of Typhoid Vaccination by Mouth, *Am J Hyg* 9: No. 1, 1 and 21, 1929

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## CLINICAL AND EXPERIMENTAL

### STUDIES IN THE ALIMENTARY TRACT OF MAN

#### I THE ATTAINMENT OF RELIABILITY IN GASTRIC RESPONSES\*†

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#### INTRODUCTION

THE great improvement in roentgenoscopic methods during the past quarter century and the increasing employment of the technic for diagnostic purposes call for a new comprehensive study of the alimentary tract in its normal activity. This is emphasized by the many recent attempts to associate definite forms of stomach and dispositions of intestine with particular physical types of humanity. For such conclusions the evidence seems to us ill substantiated and we do not propose to enter the discussion. It will be more satisfactory to make an inquiry of this nature after we shall have more precise information upon the norms presented by the alimentary tract in form, position and motility.

For our purpose the study must be made upon human beings young and healthy, living under conditions which are controlled or at least definitely known. The observations must be repeated upon the same subjects until the possibility of chance effect is certainly excluded. It must not be an investigation which is the side issue of a professional examination of alimentary tracts known or suspected to be the seat of disorder.

For a carefully planned investigation of this kind the alimentary tracts of medical men themselves are obviously the most suitable. We have had the privilege of using those of our colleagues and students, as well as our own in this analysis of anatomic expression of activity in the normal organs.

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†Studies I IV of this series form parts of a thesis for which the degree of A.M. was conferred by Western Reserve University upon Wilhelmine Kuenzel June 1928.  
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In this and succeeding articles we propose to deal with the stomachs of one hundred and forty-seven young healthy adult males repeatedly examined by both roentgenoscopy and radiography under controlled conditions. It is the repeated examination which we seek to emphasize as most informative and the accumulation of radiograms of the same individual. Eliminating the numerous serial studies from which cinematographic records have been made, our average is seven radiograms per subject in addition to the detailed records of the fluoroscopic examinations.

#### TECHNICAL METHODS

Certain technical methods and principles of examination which can be standardized in advance should be given their validation, as it were, by checking possible errors. These can be quickly enumerated.

#### *Barium Meal*

While most of our original records were made after ingestion of a 16 ounce meal consisting of 4 ounces of barium sulphate in 12 ounces of buttermilk, we have definitely assured ourselves that a smaller meal is more satisfactory. In a later communication we shall present the evidence for this, but it may at once be stated that our standard meal is now one ounce of barium administered in four ounces of sweet milk at 70° Fahrenheit. This is given between two and three hours after a light meal, namely, breakfast or lunch, while the subject is, as yet, unfatigued by the day's work. It is neither necessary nor advisable to utilize a starved stomach. There is at least an equal likelihood of obtaining a complete stomach outline with a small meal of five ounces which permits more experiments in a short time and does not nauseate the subject.

#### *Dosage*

A 5-30 M A Coolidge tube, radiator type, with a 5-inch gap is used for both roentgenoscopy and radiography, 5 milliamperes being employed for the former and 30 milliamperes for the latter. The target distance for radiography is 36 inches, the exposure one to one and a half seconds, depending on the size and weight of the subject, and the tube is centered at the level of the disc below the second lumbar vertebra. Under no circumstances, even when a serial study is undertaken for cinematographic reproduction, is the total exposure permitted to exceed half a skin dose.

#### *Tube Level*

The close relation of stomach to vertebral column effectually prevents distortion from being registered in this relationship as a result of minor modifica-

TABLE I  
INFLUENCE OF TUBE LEVEL

PATIENT	TUBE LEVEL	CARDIA	PYLORUS	GREATER CURVATURE
M R	U	MT12	UL4	Disc below L5
	L	MT12	Disc L3 L4	LL5
M E B	U	ML1	LL4	1 cm below L5
	L	UL1	LL4	1½ cm below L5
W M K	U	LT12	LL4	LL5
	L	LT12	LL4	ML5
T W T	U	Disc T12 L1	LL3	UL4
	L	UL1	LL3	UL4

U = upper M = middle L = lower

tions in tube level. In a large series involving frequent repetition of radiography on a single subject this is easily recognized. Table I is merely an example of the result obtained when the tube levels are deliberately two and a half inches apart. The discrepancy of one third of a vertebra occasionally registered is no more than an expression of difficulty in reading levels on the film.

TABLE II  
INFLUENCE OF CHANGE OF TUBE LEVEL ON VERTEBRAL COLUMN

	λ1917 E TUBE CENTERED ON L3	λ1917 F TUBE CENTERED 4 IN. HIGHER
Cardia	LT12	LT12
Pylorus	ML3	ML3
Greater Curvature	Disc L5 S1	Disc L5 S1

Table II gives the readings on a subject when the difference in the tube level was four inches. The readings were of course, made without any idea of checking. It is but a chance culling from our records.

### Errors

*Stance of Subject*—Differences in vertebral curvature are the most fruitful source of discrepancy, the effect being naturally most pronounced by modification of lumbar curve. Modification in respiratory phase has a very minor effect.

*Psychologic State of Subject*—The instability of stomach position in an untrained subject is easily demonstrated (see Routine Examination page 1021).

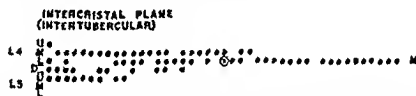


Chart I—Site of intercrystal plane in 96 male white students. Vertebrae indicated in thirds: upper, middle, lower. Disc between fourth and fifth lumbar vertebrae also plotted. Median (encircled), mean (M) and mode all at lower L4.

### Criteria of Position

*External Features*—The vertebral column is the safest guide. Each lumbar disc corresponds roughly to one third of a vertebra. Hence a vertebra and its subjacent disc form a group of four vertebral units. The intercrystal plane is distinctly less useful because its level, relative to the vertebral column, varies (Chart I), its level, read on the fluoroscopic screen, is not precisely that shown on the radiogram, variations in stance affect the site of the crest shadow just as greatly as they affect the vertebral column. The umbilicus is practically constant in site at the level of the intercrystal plane.

*Gastric Features*—Vertical length. Maximum distance projected on the film in a straight line parallel with the vertebral column, from highest limit of Magenblase to lowest point on greater curvature. Cardia pylorus and cardia greater curvature lengths are also employed. Of these absolute dimensions one can make indices but, like planimeter records of shadow area, they are of little value for computation of position since they must vary with the absolute dimensions of the subject himself. Fluoroscopic tracings are also of little value because the faint shadow is difficult to see with sufficient exactitude even



through the thinnest tracing paper practicable, it prolongs unnecessarily the exposure period, and respiratory movements, which cannot be eliminated even when the tracing is rapidly made, are reflected in a rise and fall of the stomach

#### METHODS OF PRESENTING RESULTS

Charts are useful for showing the position of various parts of the stomach relative to the vertebral column. Tracings show size, shape and motility, as well as position. Planimeter records of area permit a comparison of relative size. Of course no one of these methods can be more accurate or dependable than the radiograms from which the records were taken. No one of them throws more than a very imperfect light on any problem. But in biology we are often faced with phenomena which result from the interaction of many diverse factors. And the only method of analysis may be the removal of one factor at a time. By this slow and tedious method we attempt to glean information from our records.

On the charts a vertebra is indicated in thirds, upper (U), middle (M), and lower (L). Beneath it is the disc (D). The intercristal (in practice the same as intertubercular) plane is taken in its modal position (ascertained on our series), namely, the lower third of the fourth lumbar vertebra.

For demonstrating actual changes in a stomach the most satisfactory scheme is the superposition of successive radiograms, but no person can hold himself in exactly the same posture at two successive examinations. Straightening of the lumbar curve withdraws the vertebrae from the film, and then shadows take on greater dimensions. Increase of the lumbar curve causes the vertebrae to approach the film, and then shadows consequently diminish in dimensions. Hence superposition of radiograms is not so easy in practice as it is in theory. Again, the pelvis is often slightly tilted to one side or the other. And thirdly, the arrest of diaphragmatic movement may have occurred in slightly different phases of the respiratory cycle. If the subject be instructed to inhale deeply and then to exhale until his chest reaches the position of greatest ease, he will usually stop his diaphragm in the same or practically the same position each time. The inclination of the rib shadows on superposition permits estimation of the error, if any, from this cause.

The tracing is not an accurate indication of the vigor of gastric activity, and one must not depend upon it for an estimate of the amplitude of peristalsis. It is at best a chance momentary representation of a continuous action, and, indeed, it may show no indication whatever of peristalsis, for sometimes the very technique of radiography may temporarily inhibit gastric peristalsis. It is on the fluoroscopic study that we must rely for accurate information on peristalsis.

As for planimeter records, it may be objected that area is no criterion of volume. The directions in which a stomach enlarges are, however, quite definitely those of the longitudinal and circular muscular fibers, lateral distention taking place along the greater, never along the lesser, curvature. In spite of careful search we have never seen the stomach enlarge in its anteroposterior dimension in comparison with its enlargement in transverse or longitudinal dimensions (see Influence of Occupation page 1025).

Considerable experience is required to determine with assurance on a radiogram the outlines of cardia pylorus, lesser curvature and sometimes of gastric tube even after administration of a meal. This is not due to the smallness of the meal, the pylorus may be quite indefinitely delineated on the radiogram even after one has observed, by means of the fluoroscopic screen, barium passing into the duodenum. The sites of cardia and lesser curvatures are still more difficult to define, and resulting errors affect the planimeter record. Six or seven per cent of error in area must be allowed for (see Influence of Occupation, page 1025). In spite of these defects the planimeter record is a useful method of comparison for a particular stomach at successive examinations and with reservations it may be usefully employed in the comparison of different stomachs, especially if the subjects be young, about the same age, comparatively slender and of fairly uniform build.

#### ROUTINE EXAMINATION

All our subjects have been examined between two and three hours after a light meal, breakfast or lunch. We find that by this time the stomach is empty except for the negligible residuum which is apparently normal to some people (see Physical Condition of Subject). It is an essential feature of our study that the stomach should be empty but not starved.

Although studies have been made of our subjects in both vertical and horizontal postures we confine our attention to the former unless it is specifically otherwise stated.

By administering the experimental meal in different amounts to the same subjects we found that size of meal bears no direct relation to the exact size or precise position of the untrained stomach and even on the trained stomach it is quite without effect upon gastric shape. The untrained stomach does not adjust itself systematically to the exact amount of barium contents and is usually practically its original size after much contents have passed through the pylorus.

A fundamental necessity of all roentgenologic examinations is the quiet, orderly carrying out of the technical procedure. Any confusion or irregularity in the precise arrangements or even uncertainty in the minds of those responsible is at once transferred to the subconscious ego of the subject and is reflected in his alimentary response. Every detail of our technique is therefore carefully rehearsed by the staff before the actual session commences.

#### PHYSICAL CONDITION OF THE SUBJECT

It is not enough to safeguard one's observations by carefully controlling the methods of examination. The physical condition of the subject and his psychic state must also receive careful attention and control must be established over these features of the work.

The antiquated preliminary routine of purgation and starvation itself had profound effects on the subject both physically and psychically. Purgation is no longer required because of increased penetrating power of the radiographic apparatus. Neither is starvation necessary to empty the stomach. The subject should suffer no interruption of his daily habits. The lapse of between two and

three hours after a previous light meal such as breakfast or lunch is enough to ensure an empty stomach. If, as happens in some people, a small residuum of contents remains, it is expelled at the moment of or quickly after ingestion of the new meal.

### *Time of Day*

Since circumstances compel us to examine freshman students in the afternoon and sophomores in the morning, it is necessary to inquire whether any differences found in gastric behavior may be due simply to the time at which examination is made. To make a critical investigation of this problem on a large scale is impracticable, the evidence must be obtained by chance. We have, however, experimented with stomachs of members of the staff at all times, the same stomach being examined in early morning, at noon and in the afternoon. We have failed to find any evidence of real difference in response except in the late afternoon of a strenuous day. This difference is plainly due to fatigue and is not relevant to the present problem. On the basis of our staff experiments we believe it permissible to compare morning and early afternoon records. This decision is supported by our finding that the February afternoon records of freshmen are intermediate in type between the October afternoon freshman and October morning sophomore records (see Possible Influence of Season, page 1030).

### *Effect of Body Posture on Stomach Position*

To investigate this problem a group of students in the latter part of their freshman year submitted themselves for examination. Each was studied in both vertical and horizontal postures. Two radiograms were taken successively with the subject standing erect and lying on his back. The results are given in Table III and Chart II. Cardia, pylorus and greater curvature all undergo definite modification in site, though individual variation in the extent of this modification is considerable. Cardia is the least affected, and its change in position may be inseparable from modification in precise position of the diaphragm induced by change in posture. Concerning this we

TABLE III

CHANGE IN STOMACH POSITION WITH CHANGE FROM VERTICAL TO HORIZONTAL BODY POSTURE

PATIENT	DATE	CHANGE IN SITE IN VERTEBRAL UNITS		
		CARDIA	PYLORUS	GREATER CURVATURE
203	Jan., 1925	4	5	7
204	Jan., 1925	3	5	10
208	Jan., 1925	4	11	12
212	Jan., 1925	6	?	13
214	Jan., 1925	2	6	8
215	Jan., 1925	4	7	10
215	Oct., 1925	1	4	7
217	Jan., 1925	0	7	6
224	Jan., 1925	?	6	7
227	Jan., 1925	1	4	5
227	Oct., 1925	0	6	8
229	Jan., 1925	0	5	7
229	Oct., 1925	4	10	11
Total (9)		29	76	111
Average		3.2	8.3	12.3

have at present no data. The cardia is elevated 3.2 vertebral units, that is to say, about the height of one vertebra. The pylorus is raised approximately twice and the greater curvature almost three times this amount. Since these figures are obtained from the record of the number of vertebral units through which the gastric features rise in each individual, we feel they give a truer picture than does the chart which registers the differences between average positions of the respective gastric features before and after change of posture. By the chart the cardia rises two units, the pylorus six and the greater curvature ten.

In spite of modification in position of the three chief sites of the stomach we must emphasize the fact that the essential shape of the stomach does not change in corresponding manner. The proof of this contention lies in exami-

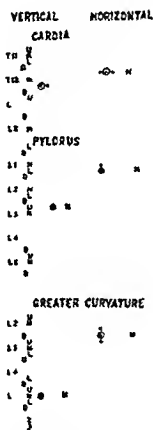


Chart II—Level of stomach sites in 14 male White students. Vertical and horizontal postures. Indicators as in Chart I.

nation of the radiographic records themselves and we can present merely one illustrative instance (Fig. 1). Student No. 215 was examined in January, 1925 and in the following October. During this interval the general gastric contour altered so that in October the stomach was more obliquely placed than in January. In the change from vertical to horizontal posture this essential difference in gastric contour is maintained. Now it must be remembered that this stomach (No. 215) was not a mere casual untrained organ. One cannot too often insist that the stomach of unpredictable response is that of the casual patient or untrained subject.

#### *Relation of Change in Weight to Stomach Position*

In making examinations involving long periods of time the problem of change of weight sometimes obtrudes itself quite emphatically and must therefore receive attention. Now the fluctuation in weight during a single

day, as shown by our staff records, may amount to or even somewhat exceed four pounds. Hence we have ignored changes in weight of less than this amount. These changes in weight may have developed during a period of six or of twelve months, the lapse of time is unimportant.

In the series utilized there were fourteen students whose weight had increased by an average of 7.3 pounds, and ten whose weight had decreased on the average 5.6 pounds. In the series with increased weight the total rise in position is recorded for the three chief sites of the stomach, having regard to sign. For the cardia this amounts to eleven vertebral units, for the pylorus twenty-seven and for the greater curvature fifty-six, approximately an average of one vertebral unit (or one-third of a vertebra) for the cardia, two for

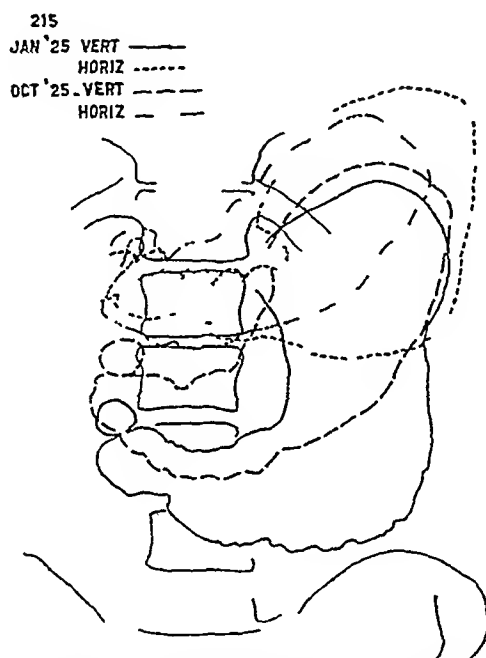


Fig 1—Effect of body posture on stomach position. Change from vertical to horizontal posture is accompanied by alteration in levels of stomach sites but not in essential characters of stomach outline.

pylorus and four (one vertebra with disc) for greater curvature. There is great individual variation and no uniformity in change of level. As the results appear so equivocal it would be quite justifiable to hold that some factor other than increased weight is responsible for the change in position even of the greater curvature.

In this uncertainty we turn to the second part of the table. The average diminution in weight of the ten students in the other group is 5 to 6 pounds. The total lowering of cardia, having regard to sign, is ten units, of pylorus twenty, and of greater curvature ten. In our routine work we should be disinclined to pay much heed to a change in position averaging for cardia and greater curvature one unit each and for pylorus only two units. But the lowering with loss of weight and raising with gain in weight occur far too uniformly for us to dismiss them as pure chance, especially since it is by no

means necessary to postulate a direct or proportional relationship between change in weight and modification of stomach site. Our results do at least seem to bear out the observation that small highly placed stomachs occur in the healthily corpulent and low placed stomachs in the thin emaciated and enfeebled.

TABLE IV

1 CHANGE IN STOMACH POSITION WITH INCREASE IN WEIGHT					
PATIENT	INCREASED WEIGHT	CHANGE IN SITE IN VERTEBRAL UNITS			
		CARDIA	PYLORUS	GREATER CURVATURE	
1005 A E	+ 4 lb	+ 1	+ 1	+ 4	
1006 A D	+11½	+ 5	+ 4	+ 1	
1009 A E	+ 4½	0	- 2	+ 3	
1010 A D	+ 4½	- 2	- 3	- 1	
1024 A D	+ 8	- 4	- 2	+ 3	
1025 A D	+ 4½	+ 1	+ 3	+ 4	
1029 A F	+10	0	0	0	
1037 A D	+ 4	- 2	+ 1	+ 4	
305 A I	+19½	+ 1	- 2	+ 6	
308 A G	+10	+ 5	+ 3	+ 5	
309 A G	+ 4	+ 1	+ 0	+ 7	
312 A G	+ 6	0	+ 1	+ 3	
317 A G	+ 7	+ 3	+ 7	+ 7	
332 A G	+ 4	0	+ 3	+ 4	
Total 14	Av + 1½	Total +11	+27	+5b	
2 CHANGE IN STOMACH POSITION WITH DECREASE IN WEIGHT					
PATIENT	DECREASED WEIGHT	CHANGE IN SITE IN VERTEBRAL UNITS			
		CARDIA	PYLORUS	GREATER CURVATURE	
1017 A E	- 8 lb	+ 1	- 7	- 7	
1021 A E	- 4	0	0	0	
1023 A E	- 5½	- 3	- 3	- 3	
1026 A E	- 7½	- 4	- 2	- 1	
1030 A D	- 4½	- 1	- 2	- 3	
1033 A D	- 6½	- 2	- 1	- 1	
311 A G	- 7½	- 2	0	- 2	
303 A G	- 5	+ 1	- 2	0	
320 A G	- 4	0	- 3	+ 5	
336 A G	- 4	0	0	+ 4	
Total 10	Av - 5½	Total -10	-20	-10	

Since the pylorus is much less susceptible than the greater curvature to direct pressure by other abdominal organs, the change in position of pylorus has a greater possible significance.

We must now consider certain conditions which may affect the subject but are even less easily segregated in their influence than those we have studied.

#### *Influence of Occupation*

It is a well known observation that the general physical condition is reflected throughout the systems. The occupation of a student, therefore, may only be considered to affect his alimentary tract in so far as that occupation affects his general health.

In two groups of our October sophomore series we have taken planimeter records of area from tracings made of stomach radiograms, separating the records according to occupation of the students.

To the first group were given 16-ounce buttermilk meals. Of these students thirteen had been occupied during the summer in indoor work such as hospital laboratory technicians, night clerks and workers in machine shops. Twenty-two came from outdoor occupations such as road engineers, camp counsellors and farmers. The complete observations are set forth in Table V. Crude average area of stomach shadow determined from tracings of radiograms made ten minutes after ingestion of the meal is 20806 sq mm for indoor workers and 18836 sq mm for outdoor workers. The difference between these figures, namely 1970 sq mm, somewhat exceeds 10 per cent of the outdoor average.

To the second group 5-ounce buttermilk meals were given. The crude average area of the eight indoor stomach shadows is 11797 sq mm whereas that of the nine outdoor stomachs is 12287 sq mm. This time the difference, 490 sq mm, is slightly more than 4 per cent of the indoor average.

TABLE V  
AREAS OF STOMACH SHADOW

16 OUNCE MEALS					
INDOOR OCCUPATION			OUTDOOR OCCUPATION		
PATIENT	AREA		PATIENT	AREA	
221F	14960	sq mm	201F	18060	sq mm
227E	23560		202E	17770	
228F	18180		204E	14700	
229E	19700		205E	18800	
230E	22900		207F	19430	
303G	20260		213F	16440	
307G	21200		215E	16230	
308G	24390		224E	16580	
315G	20620		225F	19900	
328G	27050		305I	19050	
329G	17880		306G	18330	
331G	21450		309G	20100	
333G	18330		310G	20950	
			312G	24750	
			318G	22740	
			320G	21400	
			321G	17530	
			327G	21790	
			330G	17300	
			332G	17200	
			335G	19250	
			336G	16100	
Average of 13	20806		Average of 22	18836	

5 OUNCE MEALS					
INDOOR OCCUPATION			OUTDOOR OCCUPATION		
PATIENT	AREA		PATIENT	AREA	
	1	2		1	2
214E	12040	11050 sq mm	1003D	16200	18100 sq mm
314M	11990	12000	1006D	11220	11700
1005E	10730	10450	1008D	14630	15800
1023E	12030	12170	1009D	10200	10190
1028E	10350	10100	1018E	10910	11450
1030D	14060	15260	1024D	8190	8350
1033D	12220	10400	1031E	14250	14900
1034D	10960	11000	1035E	14520	14500
			1037E	10460	12290
Average of 8	11797	11554	Average of 9	12287	13031

Since the outdoor average was the smaller in the first group and slightly the greater in the second we must presume that the differences are due to technical errors and not to occupation. Hence we made a second tracing of each of the 5 ounce series and have added the records to Table V. For the outdoor group the difference in average area from the two records is 744 sq mm. Hence differences which amount to less than 6 per cent of the total area to be compared must not be considered as significant (see Methods of Presenting Results, p. 1020).

Now the 5 ounce series easily falls within the 6 per cent probable error. This makes us doubt the wisdom of reading significance into the difference in the 16 ounce series. It may be that the larger amount of contents predisposes the record to a larger error. In the 5 ounce group the difference of average areas in our second set of tracings is 1477 sq mm, almost 13 per cent of the smaller average which happens to be that of the indoor stomachs.

If there be any difference in stomach area related to occupation, it must be sought and proved by other means than the one here adopted.

Incidentally it should be mentioned that we have found no way of checking anteroposterior depth of barium shadow. Our observations during the fluoroscopic examination assure us that it is much less subject to change than the transverse or longitudinal diameters but it would be absurd to

TABLE VI

1. EFFECT OF INDOOR OCCUPATION ON STOMACH ACTIVITY				
<i>5 Ounce Buttermilk Meal on Empty Stomach</i>				
No.	Sudden Distention	Sudden Elongation	Peristalsis	Passage Seen
314M	None	None	Immediate	Immediately
1030D	None	None	Immediate	Immediately
1033D	After 1 min	None	After 2 min	Immediately
1034D	None	None	Immediate	Immediately
<i>5 Ounce Buttermilk Meal 1 Hour After Milk Meal</i>				
214E	Immediate	Immediate	After 1 min	Immediately
1005E	Immediate	Immediate	After 1½ min	After 2 min
1023E	After 5 min	None	Immediate	After 5 min
1028E	None	None	After 2 min pulsation After 5 min waves	Immediately
2. EFFECT OF OUTDOOR OCCUPATION ON STOMACH ACTIVITY				
<i>5 Ounce Buttermilk Meal on Empty Stomach</i>				
1003D	Immediate	Immediate	Immediate pulsation After 4 min waves	After 4 min
1006D	After 1 min	After 1 min	Immediate	Immediately
1008D	Immediate	None	After 2½ min	Immediately
1024D	After 2½ min	None	After 8 min	After 1 min
1037D	None	Immediate	After 3¼ min shimmer	After 4½ min
<i>5 Ounce Buttermilk Meal 1 Hour After Milk Meal</i>				
1009E	Immediate	None	Immediate	Immediately
1018E	None	None	Immediate	Immediately
1031E	None	Immediate	Immediate	Immediately
1035E	None	None	Immediate	Immediately



contend that there is no alteration (see Methods of Presenting Results, page 1020)

Area of shadow, however, is but one criterion of gastric activity. One must also consider the vigor of peristalsis, and this cannot be deduced from the radiogram (see Methods of Presenting Results, page 1020). Extracts from the verbal records of the fluoroscopic examinations are submitted as Table VI. Lest an unforeseen difficulty be introduced, we have separated each series into two groups: in the first, the buttermilk (5 ounce meal) is administered on an empty stomach, in the second it is given one hour after a similar meal of sweet milk. The reason for adopting these alternative methods of exhibition of buttermilk is explained in our forthcoming article on the gastric response to milk and buttermilk. It is apparent from Table VI that any differences in response are bound up with some obscure factor in the individual himself and not due to occupation or physical condition of the stomach. We are therefore now in a position to make this further analysis of the subject himself.

### *The Group Response*

In considering the results presented in this section of our study, there occurs the thought that part, at least, of the differences observed in the October and February records may be seasonal in character. The investigation would then be incomplete without some consideration of this possibility.

In spite of the fact that we have been unable to discern any essential difference between the gastric responses of sophomores when investigated in their relation to occupation, it is equally certain that an improvement in gastric response is observed in sophomores generally, and inasmuch as this appears fairly uniformly in the group and not erratically with great individual discrepancies, we will temporarily define it as the group response. In order to render accessible the data upon which this conception is based we have arranged the planimeter records of successive groups of students in Table VII.

In the first part of this table are the average records for freshmen and sophomores made from tracings of radiograms taken ten minutes after the barium meal. The average reduction in area from freshman to sophomore years in 1925 was 3382 sq mm. It should be noted that the freshman records in this group were made in February, not in October, of the freshman year. They were the first records of students made in this investigation. It is, of course, impossible to decide whether the smaller average area in the student as a sophomore is due to more rapid emptying or to a smaller increase in size on swallowing the meal than is characteristic in freshmen. At the moment we are concerned with the record of size alone.

The next group of students was examined at the beginning of their freshman and sophomore courses in October 1925 and October 1926. In addition they were examined in February 1926. The decrease from October to October was only 2379 sq mm, and that from February to October 2450 sq mm. Let us ignore for the moment the fact that the change is equally great from February to October as from October to October, and also the

fact that the reduction in area in this group is less than that shown in the first group. The important fact is that there is a reduction.

Another phase of this same investigation is shown in the second part of Table VII where we observe the reduction in area of stomach shadow between the taking of the 10 minute and 60 minute radiograms after the meal. In the freshman of February 1925 the reduction of area is 4202 sq mm. In the same stomachs the following October the reduction is 4442 sq mm. In the succeeding group, the freshman October diminution was 3916, the freshman February diminution was 4823, the sophomore October reduction was 5760 sq mm.

TABLE VII  
COMPARISON OF FRESHMAN AND SOPHOMORE AREAS

RECORDS FROM RADIOGRAMS TAKEN 10 MINUTES AFTER MEAL					
		Year	Average Area		Difference
Class of 1928					
Freshmen	(A)	Feb 1925	21545 sq mm		
Sophomores	(A <sub>2</sub> )	Oct 1925	18163		3382 (A <sub>1</sub> -A <sub>2</sub> )
Class of 1929					
Freshmen	(B <sub>1</sub> )	Oct 1929	22829		
Freshmen	(B <sub>2</sub> )	Feb 1926	22900		2319 (B <sub>2</sub> -B <sub>1</sub> )
Sophomores	(B)	Oct 1926	20450		2450 (B <sub>2</sub> -B <sub>1</sub> )
RECORD OF REDUCTION IN AREA FROM 10 MINUTES TO 1 HOUR AFTER MEAL					
		Year	At 10 Min Area	At 1 Hr Area	Difference
Class of 1928					
Freshmen		Feb 1925	21545 sq mm	17343 sq mm	4202 sq mm
Sophomores		Oct 1925	18163	13720	4442
Class of 1929					
Freshmen		Oct, 1929	22829	18913	3916
Freshmen		Feb, 1926	22900	18077	4823
Sophomores		Oct 1926	20450	14690	5760

The second part of the table detailing the reduction in area during the hour, perhaps points to the inference that the reduction in area when freshman and sophomore 10 minute radiograms are compared is due rather to a more rapid emptying than to a smaller initial increase in size. Whichever be the true explanation the striking fact remains that a significant decrease is noted. Some students exemplify this better than others, but it occurs in the group as a whole and is associated with progressively increased experience and more understanding cooperation. In other words, it is associated with an increasingly stable and controlled central nervous reaction. We believe that any experience or condition of life which increases the stability and control of the central nervous system improves also the gastric response in the manner just indicated. While we have been unable to determine from quantitative analysis any relative difference in improvement as between freshman and sophomore responses which can be related to the type of life which the individual has been living, we still feel that the gastric response of a student who has been leading a healthy and wholesome out of door existence is better than that of a student occupied in indoor or dismal and un congenial surroundings.

*The Possible Influence of Season*

The first part of Table VII shows that reduction in area of the 10-minute radiogram is to be found only in the sophomore stage there is no difference between the average areas of stomach shadow recorded in October and February of the freshman year. Nevertheless the 60-minute radiograms demonstrate a more marked reduction in area of shadow during the hour in February when the students have grown accustomed to our routine. It came as a surprise to us to find that the 10-minute radiograms showed an average area as large in February as in the previous October. The group response which upon the basis of the previous year's work (Table VII, A<sub>1</sub>, A<sub>2</sub>) we felt sure would be evident, and which indeed is evident in the 60 minute radiograms, is utterly lacking in the 10-minute ones.

There are two interpretations possible from these records. Either increased gastric vigor in the February records shows itself as more rapid emptying which is tardy in its full development, or there is some influence inhibiting the stomach from that immediate activity characteristic of the October sophomore. If the former explanation hold good we might expect to find progressively improved gastric activity in the February examinations as the years pass and the tradition of this method of instruction becomes better established. We have just completed the spring studies of the 1931 class and although the records are not yet in order for quantitative analysis, we find, to our disappointment that no obvious improvement is evident over the records obtained of the same stomachs last October. Apparently then the group response cannot be depended upon to act early after ingestion of a meal in February of the freshman year. However, since it is obviously present in the 60 minute radiograms, there must be some factor inhibiting its expression at first. Further, so far as we are able to judge, this factor is equally as potent now as it was two years ago. The possible factors which one might invoke are occupation and season. After a few months of the confined and close study required by the medical curriculum it might be expected that the gastric response would be modified. But we have already presented our data upon this score and have failed to demonstrate quantitatively any effect of occupation upon gastric response.

The factor which we have not yet considered is that of season. It would be reasonable to assume that even all the varied influences which can be grouped under the term season would be able to curb but imperfectly the improving gastric response. As a working hypothesis we present this aspect of our study in the expectation that opportunity will occur for later and fuller investigation. Such scraps of information as we have been able to elicit from our observations on stomachs of members of the staff and other chance opportunities presented to us, all point in the same direction. They suggest that gastric activity is more sluggish during the winter months, and that most uniform responses are to be elicited after the health-giving, greater freedom of the summer.

It must be noted that the term season is being used in a very broad sense, covering all living conditions, including diet, reduced bodily activity, occu-

pational conditions, lack of sunshine and fresh air, which are regularly associated with winter in this latitude

#### CONCLUSION

It is at this stage of the investigation that we find solid ground beneath our feet for the first time. The fault from which all previous work on the alimentary tract suffers is the overlooking of the necessity to use only trained stomachs under adequately controlled conditions. Failure to secure this essential basis is responsible for the bizarre, conflicting and equivocal results hitherto obtained. From the day when this secret was revealed to us our investigations have taken on a new directness, and the results have assumed that orderliness which one may expect from a properly controlled physiologic experiment. It is for this reason that we have developed the argument in this article up to the stage of the group response. In further communications it is possible to present the results of our studies upon the normal motility of the human stomach.

This article is a record of our effort to find a secure basis upon which to build a new study of the normal motility of the human stomach.

It is clear that uniform controlled or known conditions must be secured with far greater certainty than has hitherto been attempted before results of any real value can be obtained.

We have made it our business to investigate critically each step of the necessary procedure and have ultimately discovered in the subject himself the source of error which has constantly vitiated all previous work. Every other factor can be allowed for except this. But the reaction due to this factor is incalculable. The subject must be willing, capable of entering intelligently into cooperation with the observer, and he must be trained so that the subconscious effect upon him of the roentgenologic examination is at a minimum. Inadequate appreciation of the essential nature of this precaution will certainly lead to disaster.

In our investigation of the problem we have standardized our technique and our methods of presenting results. We then set out to standardize our subjects, controlling time of day, body posture, weight, occupation and season of year. It is after a full year's training in roentgenologic method and general medicine that the student first becomes reliable as a subject. Until that date his reactions cannot be predicted with any assurance. Hereafter we shall demonstrate how easily they may be upset even when once established.

#### SUMMARY

1. An investigation of the human alimentary tract which does not involve standardized technique, controlled conditions of experiment and subjects carefully trained in roentgenology, is nowadays without value.

2. The old large meal on a fasting stomach is less likely to give a truly normal result than a small meal upon an empty but not starved stomach.

3. The close anatomic relationship between stomach and vertebral column in the living makes the latter the best indicator of gastric position. The exact level of an x-ray tube is of little importance in determination of site of gastric points.

4 Planimeter records of area are to be accepted with reserve, for they may result in considerable error if used to indicate gastric volume. Properly controlled and used as averages of series they can be quite helpful.

5 Time of day within reason has no effect upon gastric response. Time of day which really involves fatigue has a marked effect. Season of year probably has an effect, although an indirect one.

6 Alteration of body posture influences levels of gastric features but not essential characters of gastric contour.

7 Change of body weight seems capable of an indirect influence on gastric shape exerted through the general well-being of the subject. Occupation acts in the same manner.

8 The culmination of our work lies in the demonstration of the influence of training of the subject upon constancy of gastric response. Further studies amplify this fundamental necessity of all gastric investigations.

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## CORYNEBACTERIUM ULCERANS ITS EPIDEMIOLOGIC IMPORTANCE\*

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BY RUTH GILBERT, A.M., M.D. AND F. CONSTANCE STEWART, M.S., M.D.,  
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**P**ATHOGENIC microorganisms resembling diphtheria bacilli in morphology are always of interest and especially when they are found in lesions of the throat, since the clinical manifestations may be attributed to diphtheria and microscopic examination of cultures may offer no assistance in determining the etiology of the infection. We have been studying a species of such microorganism for which the name *C. ulcerans* has been proposed. Even though cultures of these bacteria are not encountered frequently, they have been found in specimens submitted from widely separated parts of the state.

As the species has been previously described,<sup>1</sup> only the characteristics which have seemed most important need be mentioned. In young cultures, after from four to eight hours' incubation, these microorganisms have the morphology of diphtheria bacilli. In older cultures, following from eighteen to twenty-four hours' incubation, the majority of the cells are coccoid. More of the bacillary forms may be found, however, in mixed cultures after the longer incubation period. Culturally, this species resembles the diphtheria bacillus, except in its ability to liquefy gelatin and its failure to reduce nitrates. One of the most striking things concerning the microorganism is the extremely slight protection which diphtheria antitoxin affords animals inoculated with

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the culture or its soluble toxin. When two guinea pigs, one of which has received 500 units of diphtheria antitoxin, are given intracutaneous injections of this material, a similar reaction is induced in both animals, i.e., marked induration, congestion, and necrosis usually followed by the formation of an ulcer. After subcutaneous inoculation, a large ulcer develops and occasionally the animals die. At autopsy, the lesions are found to differ in some respects from those induced by diphtheria toxin, especially in that intense, diffuse, hemor-

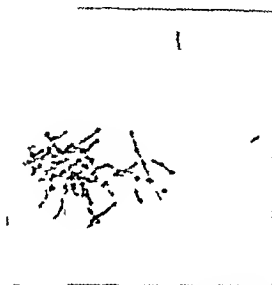


Fig 1—Four hour culture of *C. ulcerans* on Loeffler's blood serum medium demonstrating polar bodies. Albert's stain. X2000 diameters.



Fig 2—Four hour culture of *C. ulcerans* on Loeffler's blood serum medium showing banded forms. Albert's stain. X2000 diameters.

rhagic infiltration of the adrenals is absent. Like diphtheria bacilli, the microorganisms remain localized. They have not been found in the heart's blood.

Owing to the marked changes in morphology which occur during incubation, the presence of *C. ulcerans* in throat cultures is seldom recognized if a morphologic examination only is made. When bacillary cells are found, the cultures are thought to contain diphtheria bacilli or organisms resembling them, while the short forms may be mistaken for cocci.

In the past four years there have been three outbreaks in which the clinical symptoms have in some cases simulated diphtheria. *C. ulcerans* has been

associated with these infections, although the presence of diphtheria bacilli could not be definitely excluded. From September 4 to 29, 1925, 16 cases of diphtheria were reported in the vicinity of a county laboratory.\* Although the clinical symptoms were not considered entirely typical, the appearance of the throat warranted the administration of antitoxin. No diphtheria bacilli were found in most of the first cultures, but they were reported in many of the second. In an attempt to learn the reason for unsatisfactory results, the bacteriologist in charge of the laboratory collected a series of cultures himself and examined them with equally irregular results. None of the organisms associated with Vincent's angina was recognized. Furthermore, when cultures from some of the patients were examined in a near-by city laboratory, no diphtheria bacilli were found.

One of the cases which occurred in the epidemic proved fatal. Four days before death, a physician saw the patient and prescribed a gargle. A culture was not taken. The day before death, another physician was called. At that time there was a membrane extending over the uvula and soft palate and into the mouth. A diagnosis of diphtheria was made, 12,000 units of antitoxin were administered and a culture was taken. Prior to the death of the child on the following day, an additional dose of 18,000 units of antitoxin was given. The culture from this patient was not found to contain diphtheria bacilli, but in a transfer from it microorganisms having the morphology of diphtheria bacilli were seen. When an attempt was made to secure these bacteria in pure culture by the inoculation of rabbit-blood agar in plates, all of the colonies fished were thought to contain cocci. Transfers from two of these colonies on Loeffler's serum medium, however, contained what appeared to be diphtheria bacilli. When medium in plates was inoculated with them, the bacteriologist could find nothing but coccus-like forms in the colonies studied and unfortunately the cultures were then discarded. As twelve of the patients had used milk from one dairy, cultures were obtained from all of the milk handlers. Microorganisms having the morphology of diphtheria bacilli were found in one of these cultures, which was from M. J., a schoolboy who worked part-time at the dairy, washing bottles and occasionally helping to cap them. No history of sore throat or other illness could be obtained from him. In attempting to isolate the bacillary forms in the culture from this boy, the bacteriologist encountered the same difficulty that he had experienced in studying the culture from the fatal case. Another specimen was then collected from M. J. and submitted to the laboratory in Albany. It was found to contain *C. ulcerans*. Although there is no definite proof that this species was the etiologic factor in this epidemic, the description of the laboratory findings strongly suggests that the organism was present in some instances at least.

There have been two other groups of cases in which *C. ulcerans* has appeared to have definite epidemiologic importance. In the first group, this microorganism was found in the throat cultures of five people working on a dairy farm. After examination of the first culture, the milk was very promptly pasteurized and the infection did not spread further. On June 14, 1926, a man consulted a

\*Information relative to this epidemic was received from Dr. J. P. Garen, formerly in charge of the Cattaraugus County Laboratory, Olean, October 19, 1925.

physician concerning an infected finger. The following day he complained of sore throat and in the culture taken at that time microorganisms having the morphology of diphtheria bacilli were present. When a subsequent culture was submitted for a virulence test, it was found to contain *C. ulcerans*. The clinical symptoms in this case lasted only four days. Cultures from four of his associates, none of whom had complained of sore throat contained the same species of microorganism.

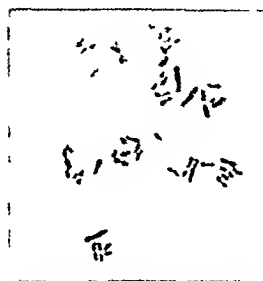


Fig 3—Four hour culture of *C. ulcerans* in infusion broth medium showing clubbed forms. Stained with Loeffler's methylene blue. X2000 diameters.

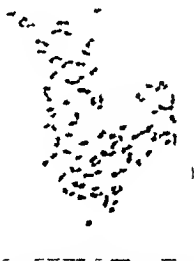


Fig 4—Forty eight hour culture of *C. ulcerans* on blood agar showing coccoid form. Stain 1 with Loeffler's methylene blue. X 600 diameters.

In the third group of cases, the infectious agent was probably derived from milk. On July 9, 1926, J. P., a boy of eleven years of age, who helped strain the milk on his father's dairy farm developed a sore throat. Although only a small amount of exudate was present on his tonsils, he was given 3,000 units of diphtheria antitoxin. No membrane was noted in his throat on the next day, but he stopped handling the milk at that time. *C. ulcerans* was found in the cultures submitted from this patient and persisted for about five weeks. From June 30 to July 31, cultures were sent for laboratory examination from 23 people who, with one exception, had used milk from the dairy farm on which J. P. was working. All had complained of sore throat. In



many of the cases, a membrane was present on the tonsils and antitoxin was administered. The symptoms lasted usually from two to three days only. *C. ulcerans* was found in the specimens from eight of these patients, but microorganisms with the morphology of diphtheria bacilli were not found in the cultures from the others. Only morphologic examinations were made unless bacillary forms were seen in the cultures. Hence some of them may have contained *C. ulcerans* which had assumed the coccoid form. From the dates of onset of the various cases, it seems probable that there was more than one carrier among the milk handlers, or that some of the patients had contracted the infection from previous cases.

It will be noted that these groups of cases have been associated with milk supplies. Most of the patients from whose cultures *C. ulcerans* has been isolated have lived in rural districts, and not infrequently have been milk handlers. The possibility of these microorganisms being derived from lesions in domestic animals, especially cows, is thus suggested. Although cultures of *C. ulcerans* have not been found to correspond exactly with the description given of any of the pathogenic diphtheroids that have been isolated from animal lesions, they resemble in many respects *C. pseudotuberculosis* of Preisz.<sup>2</sup> A comparative study of the immunologic properties of this species and other strains that have been found pathogenic for animals is therefore indicated.

#### SUMMARY AND CONCLUSIONS

The close resemblance of *Corynebacterium ulcerans* in young cultures to the diphtheria bacillus, and its coccoid appearance after longer incubation, make animal inoculation necessary for identification. Since most throat cultures receive a morphologic examination only, the prevalence of infections due to *C. ulcerans* may be greater than past experience has indicated. It is desirable to consider the possible presence of this species in cases resembling diphtheria when the results of either the clinical or the laboratory examinations are not entirely conclusive.

#### REFERENCES

- 1 Gilbert, R., and Stewart, F. C. *Corynebacterium Ulcerans*. A Pathogenic Microorganism Resembling *C. Diphtheriae*, J. LAB. & CLIN. MED. 12: 756, 1927.
- 2 Preisz, H. Recherches comparatives sur les pseudotuberculoses bacillaires et une nouvelle espèce de pseudotuberculose, Ann. de l'Inst. Pasteur 8: 231, 1894.



The change could be accounted for on the basis of photochemical sensitization. Color often develops in the absence of sunlight which would seem to exclude such a possibility. Hematoporphyrin is the only photochemical sensitizer so far known to occur in the animal body. This substance has not been detected in our work.

An aqueous solution of sodium chloride to which the silver nitrate solution is added shows a rapid reduction of the silver chloride. However, if sodium tungstate and sulphuric acid be added to the sodium chloride solution before the addition of the silver nitrate, reduction does not take place even in direct sunlight. In the protein-free filtrate of normal blood made with sodium tungstate and sulphuric acid some element is present which protects the silver chloride against reduction. In the filtrate showing the marked color changes this protection is lost. It seems probable that a loss of inhibition of reduction occurs in upper gastrointestinal tract obstruction.

Certain cross experiments have been made with washed corpuscles and plasma both from normal blood and from blood showing marked color changes. The results have not been entirely uniform. The filtrate from the mixed plasma and washed corpuscles from a positive blood which separately give no reaction, gives the characteristic color changes. The filtrate from washed corpuscles of positive blood mixed with plasma from a normal control also shows color changes. Usually the filtrate from washed corpuscles of control blood mixed with plasma from positive blood shows no color changes in the precipitated silver chloride. These experiments indicate that the color reaction is dependent upon elements in both cells and plasma. The elements in the cells are specific for the change characteristic of upper gastrointestinal tract obstruction. The element supplied by the plasma seems to be nonspecific since normal plasma works equally well.

After blood filtrates have stood overnight in the ice box usually no changes are observed even when the variations in color were most marked in fresh preparations. Precipitates which show marked color changes, after standing a few hours, often return to the normal white color.

The phenomenon described here is of interest as giving possibly some clue to the toxemia characteristic of upper gastrointestinal tract obstruction.

#### SUMMARY AND CONCLUSIONS

The silver chloride precipitate in chloride determinations in the blood of the dog after upper intestinal tract obstruction is often rapidly reduced with the production of a purple, rose or maroon color.

Such color changes are not observed in chloride determinations on the blood of normal animals.

The color changes do not parallel the rise in nonprotein nitrogen, the chloride level, or the clinical signs of toxemia.

Filtrates made from the plasma show no color changes while filtrates made from the unwashed cells do show such a change.

After washing the cells with physiologic saline solution color changes are no longer apparent unless fresh plasma be added.

The exact cause of the phenomenon is not apparent.

## THE NOSTRIL REFLEX IN LUNG DISEASE\*

BY HAL LIELEP M.D., PASADENA, CALIF.

SOME years ago F. M. Pottenger called attention to certain physical signs which attended pulmonary tuberculosis. Atrophy of the skin and subcutaneous tissues, muscle rigidity and atrophy were found to be valuable criteria in estimating the extent of pulmonary disease. Later he observed that the same laws held true in diseases of the abdominal cavity. The sympathetic reflex arc between deep organs and surface structures has been well worked out, and its value as a diagnostic and prognostic aid can scarcely be estimated.

This communication presents evidence which indicates that there is probably a reflex arc between the air-cell capacity of the lungs and the size of the nostrils which Dr. Pottenger thinks is a vagus reflex. A few years ago while attending a case of far advanced pulmonary tuberculosis I noticed that as the end gradually approached the patient's nostrils collapsed more and more. During the last few weeks the left nostril collapsed completely and post-mortem showed little or no alveolar tissue present in the left lung. Since that time every case I have had the opportunity of observing seemed to show a definite relation between nostril size and alveolar cell destruction always on corresponding sides of the body but it was not until lately that the time and material at hand permitted a closer and more detailed study.

The first series of cases were studied in the tuberculosis wards at the Los Angeles County Hospital. The nostrils were carefully sketched, Pottenger's signs noted and any additional information that the patient could supply was added. After this series of cases were studied it was decided to examine more cases under better conditions so the work was carried to Olive View Sanatorium at San Fernando, California. There it was not only possible to make very careful bedside examinations with the aid of the house physicians but also to check our nostril findings with the chest films. This was a distinct advantage.

During this study it was also noted that cases of chronic fibroid phthisis especially the ones that showed clubbing of the fingers and toes also showed a thickening of the nasal tissues surrounding the nostrils.

There are certain cases which at first seem to be exceptions to the rule but a careful observation makes it comparatively easy to differentiate them. These are the cases showing nasal deformity from early rickets. There is not only nasal septum deflection but even deformity of shape and size of the sinuses. The nostrils in these cases at first seem unequal but when carefully measured prove to be the same size, as long as no pulmonary complication exists although their respective shapes are not identical.

\*Read before Trudeau Society of Los Angeles January 23, 1933.  
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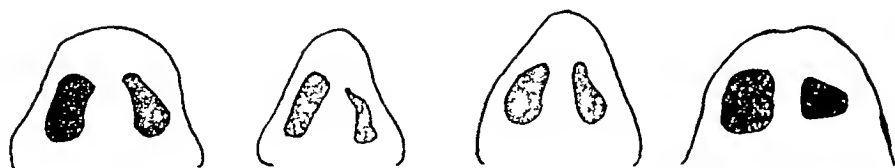


Fig 1

Fig 2

Fig 3

Fig 4

Fig 1—Upper Negro male aged twenty-two one month in bed Muscle spasm and atrophy over left chest. Lower Negro male aged seventy-four sick many years Slight atrophy and muscle rigidity over right chest

Fig 2—Upper White male aged thirty-eight nine months in bed Marked atrophy and muscle spasm over left chest States cavity in left lung Lower White male aged forty two years in bed Much atrophy of right chest right sternomastoid muscle spastic.

Fig 3—Upper White male aged forty-eight seven months in bed Atrophy of left chest a much depressed supraclavicular fossa interspaces sunken and muscles rigid Lower Negro male aged fifty-three, three years in bed Left chest atrophic painful left sternomastoid spastic

Fig 4—Upper Negro male aged twenty-one one year in bed Atrophy above and below left clavicle Lower White male aged twenty-eight five years in bed Whole chest markedly atrophic States large cavity in left lung



Fig 5

Fig 6

Fig 7

Fig 8

Fig 5—Upper Negro male aged forty-two two years sick. Right chest shows muscle spasm and atrophy Lower White male aged thirty-one nine months sick Apex sunken and marked atrophy on left side.

Fig 6—White female aged twenty-six five years sick Dotted lines show approximate shape of nostrils eight years ago Left pneumothorax fluid at base

Fig 7—White female aged forty eight years sick

Fig 8—White female aged twenty-four four years in bed

The illustrations are self explanatory, so only a few notes have been appended. Figs 1 to 5 show the open air ward cases at the Los Angeles General Hospital. Figs 6 to 19 show cases studied at Olive View Sanatorium. The x ray findings are given diagrammatically, the shading to note degrees of fibrosis, the circles cavities, fluid as dotted areas and normal lung tissue as unshaded. Figs 20 to 23 show cases of bronchiectasis. It was most interest



Fig 9

Fig 10

Fig 11

Fig 1

Fig 9—White female aged twenty eight six years sick

Fig 10—White female aged thirty one year in bed

Fig 11—White female aged forty one two years in bed Dense nodular infiltration equally distributed from apex to base. Large apical cavity with fluid level

Fig 1—White female aged thirty six three years in bed Pneumothorax on left

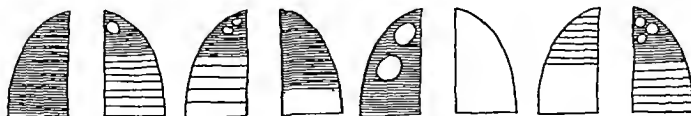


Fig 13

Fig 14

Fig 1

Fig 16

Fig 13—White female aged thirty one year in bed Right lung shows very fine fibrosis throughout. Left lung a dense mottling

Fig 14—White female aged twenty ten months in bed Heart pushed over to right Left lung shows thick walled pleurae also effusion

Fig 15—White female aged thirty five one and one-half years in bed Right pneumothorax

Fig 16—White female aged twenty eight, one and one-half years in bed

ing that none of the bronchiectatic cases showed the nostril reflex Fig 24 shows a case of actinomycosis of the lung which was kindly offered to me for study by Dr George Dock of Pasadena In this case physical examination disclosed that much more of the lung was involved than the x-ray film indicated

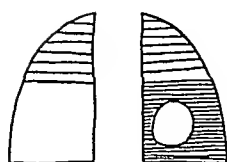


Fig 17

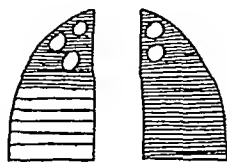


Fig 18

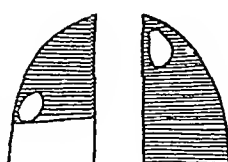


Fig 19

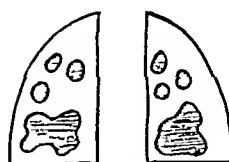


Fig 20

Fig 17—White female aged thirty-eight one and one-half years sick

Fig 18—White female aged twenty-one three years in bed

Fig 19—White female aged twenty-one three years in bed

Fig 20—White male aged fifty-two deceased Coughed for ten years Eight attacks of pneumonia (twice double) Copious fetid expectoration

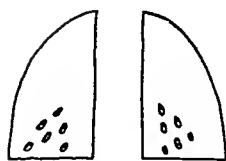


Fig 21

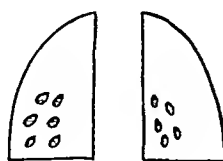


Fig 22

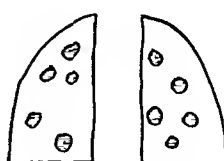


Fig 23



Fig 24

Fig 21—White female aged sixteen coughed three years Pneumonia twice.

Fig 22—White female aged thirty coughed five years Three attacks pneumonia

Fig 23—White female aged seven coughed since a baby Two attacks of pneumonia One and one-half cups purulent sputum daily

Fig 24—White male aged 50 Duration probably five years

### CONCLUSIONS

1 The nostril opening bears a relation in size to the alveolar air capacity of the lung on the corresponding side

2 Probably the reflex does not occur in uncomplicated cases of bronchiectasis. This point may have some importance in differential diagnosis between pulmonary tuberculosis and bronchiectasis.

3 As pulmonary destruction progresses the nostrils, always on sides corresponding to the lungs involved, show atrophy.

#### REFERENCE

Pottenger F M    The Symptoms of Visceral Disease 1925    St. Louis, C V Mosby Co

## BACTEREMIA DUE TO *EBERTHELLA BENTOLENSIS*\*

### WITH NECROPSY REPORT

By ROBERT A. KILDUFFE† A M, M D, WALTER B. STEWART,‡ M D  
AND WILLIAM W. HERSONH ATLANTIC CITY N J

WHILE bacteria in general have long been grouped, in their relation to disease, into pathogenic and nonpathogenic types, it is well recognized that the production of disease as a result of bacterial activities is a phenomenon influenced by a variety of factors involving the virulence and aggressiveness of the bacterium and the resisting powers of the host.

Organisms ordinarily saprophytic may invade the host in such numbers for example, as to overwhelm the normal mechanism of defense, or, when the invasion is numerically moderate, the virulence may be so high, or the resisting powers of the patient so ineffective that through such or other varied combinations of these as well as other factors the production of the phenomena characteristic of pathology and disease may follow.

The somewhat loose differentiation of bacteria into pathogens and nonpathogens is, therefore, a flexible division useful as indicating the ordinary ability of bacteria to produce harmful effects.

Exceptions to the rule are of some interest, especially when the organism in question is regarded as essentially a harmless saprophyte and for this reason the present cases of blood stream infection are reported.

The clinical and necropsy details of the first case in which this organism was etiologically concerned follow.

CASE 1—E. I., male 40 years old was admitted to the Atlantic City Hospital August 11, 1926 on the Medical Service of Drs. E. C. Chew and C. L. Andrews to whom thanks are due for permission to utilize the case for report.

Chief complaint was chills, fever, and vomiting.

*History of Present Illness*—Two weeks before admission the patient had attacks of chills and fever having at least one chill a day. He continued to work, however and four days before admission consulted a physician who made a diagnosis of malaria and administered

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a large dose of quinine which precipitated attacks of vomiting that continued until his admission to the hospital

*Past Medical History*—The patient has been an actor and professional billiard player keeping very irregular hours, eating irregularly, and working mostly at night. For the past two months he has been an entertainer at a night club.

Fourteen years ago he was in Panama, and since then at irregular intervals has had attacks of chills and fever lasting a day or so and always clearing up promptly upon dietary regulation and intestinal elimination. He has never been free from such attacks for longer than a year and usually not so long.

*Physical Examination*—On admission the patient is prostrated and jaundiced with a temperature of 105° F and a rapid pulse of Corrigan type. The blood pressure was 90/30 in the left, and 90/0 in the right arm.

He presents the physical signs of acute regurgitation and myocardial weakness. There are no rose spots, nor is there any enlargement of the spleen. The physical signs in the lungs are not distinctive.

Hb 80 per cent, RBC 4,130,000, WBC 6,200

Differential count Polys 72 per cent, small mononuclear 23 per cent, large mononuclear 5 per cent. Leucocyte index 2.4

Repeated examinations for malaria were negative, nor could clinical, bacteriologic, nor serologic evidence of typhoid, paratyphoid, or dysentery infection be found after repeated examinations during the course of the disease.

A blood culture on admission, as well as several succeeding blood cultures, gave a pure growth of a gram negative, motile bacillus with characteristics to be described below.

The course of the disease was progressive and uninfluenced by treatment, including the intravenous administration of moreurochrome 220 soluble and metaphen. The temperature ranged from 105° to 96°, the high levels being accompanied by chills, the lower by profuse sweating and profound prostration.

The leucocyte count was never over 11,500, with 87 per cent neutrophiles, and more often approximated 6,000, with 78 per cent neutrophiles.

The patient gradually became progressively weaker and more emaciated and extremely anemic. Death occurred September 2, 1926, three weeks after admission.

The report of the autopsy, performed a few hours after death may be thus summarized.

The body is that of an adult white male, much emaciated and presenting a well marked jaundice. There are no external marks of interest.

With the exception of a moderate degree of pulmonary congestion and the presence of palpable glands at the hilus, there are no gross abnormalities of the lungs. There is no evidence of tuberculosis.

The pericardial sac contains about 50 cc of blood stained serous fluid. The heart is not perceptibly enlarged and is covered with a furry, fibrinous exudate. There is an apical adhesion to the pericardial sac. The left ventricle is slightly dilated and the myocardium as a whole is somewhat soft and tears easily.

There is an enormous vegetation (about the size of a walnut) on the aortic valve, and there are numerous small vegetations on the mitral valve which is somewhat thickened.

The stomach and intestines are greatly distended with gas. The liver is slightly enlarged and definitely congested but without evidence of hypertrophy, abscess, or other pathology. The gall bladder is normal.

The spleen is soft, flabby, intensely congested, and about four times the normal size.

The kidneys are grossly normal. The intestines show no lesions of interest except a few small hemorrhagic areas in the lower portion of the ileum.

The cultural characteristics of the organism found in pure culture in the blood on several occasions before death and obtained from the blood, pericardial fluid, peritoneal fluid, liver, and spleen at postmortem were as follows.

Colonies on blood agar and cultures on plain agar slants are colorless or faintly grayish and somewhat moist in appearance suggesting somewhat those of the Friedlander group but without their viscosity

The colonies show no tendency to spread or become confluent

The organism is aerobic and grows best at 37 to 38 C Gelatin is not liquefied and a uniform turbidity is produced in broth without the formation of a pellicle Indol is formed and acid without coagulation is produced in milk

The fermentation reactions are shown in the table

	ACID	GAS
Dextrose	+	0
Lerulose	+	0
Galactose	+	0
Maltose	+	0
Lactose	+	0
Sucrose	+	0
Raffinose	+	0
Inisotol	+	0
Mannitol	+	0
Dulcitol	+	0
Salicin	+	0
Glycerol	+	0
Inulin	+	0
Xylose	+	0

Cultures were not agglutinated by *B typhosus* *B paratyphosus* A *B paratyphosus* B *B enteritidis* *B coli* or *B dysenteriae* agglutinating sera Unfortunately the patient's serum was not tested for agglutinins for this organism

On the basis of the findings above the organism was identified as *Eberthella bentolensis* (Castellani and Chambers)

Cultures taken at postmortem resulted as follows

Pericardial fluid *E bentolensis* and *B coli* right ventricle *E bentolensis* and *B coli* liver *E bentolensis* spleen *E bentolensis* peritoneal fluid *E bentolensis* *B coli* and *Staphylococcus aureus* valvular vegetations *E bentolensis* and *B coli*

From the findings above noted there is little reason to doubt the etiologic relationship of *E bentolensis* to this patient's illness and death Whether or not the recurring chills and fever noted in the history were associated with repeated invasions of the blood stream by this organism residing in the vegetations as foci or from the liver or spleen as a focus, it is of course, impossible to determine They remain, however, as logical possibilities

The second case in which this organism was of apparent etiologic importance occurred on the service of Dr Walter B Stewart

CASE 2—E J, female 8 months old admitted May 10 1928 on the tenth day of illness The attack began with vomiting (once at onset) rhinitis and cough dry and unproductive Since the third day there was an increasing fever ranging from 103 to 104 There was drowsiness fretfulness and rapid moaning respiration

On admission the physical examination showed a well nourished child weighing 15 pounds semistuporous with Cheyne Stokes respiration Except for the presence of fine crackles at the end of deep inspiration the lungs presented no significant findings

The leucocyte count was 9500 with 54 per cent polys and 40 per cent lymphocytes RBC 3200000 Hb 64 per cent Lumbar puncture gave a clear fluid without increased pressure and a cell count of 3 per c mm.

The provisional diagnosis was acute diffuse bronchopneumonia

The temperature remained between 103 and 105 until the eighteenth day of the

illness when there was a temporary drop to 101.6°, and when the patient left the hospital on the twenty first day after her parents signed a release, it was still 102° and 103° and did not finally become normal until the twenty sixth day of the illness.

By the twelfth day of the illness the respirations had become normal, but peculiar attacks of hyperesthesia were noted, the slightest sound or movement provoking irregular jerking of the head and extremities after which deep stupor occurred. This was seen only on this day.

At this time the spleen became definitely palpable, the enlargement disappearing within a week.

A radiogram of the chest on admission was reported as follows. In the left chest, about the level of the second rib, there is a small area of increased density, hardly dense enough to be pneumonia. The remainder of the lungs is comparatively clear.

One week later a further report was made. The hilum on both sides shows considerable glandular hyperplasia, and there is an area of density extending from the upper part of the root of the right lung, nodular in character, which is the result of infection of some sort. The appearance of the chest does not suggest milary tuberculosis.

The stools, at first, were watery, yellow, frequent, and contained mucus.

On the fourteenth day numerous small dull red macules resembling rose spots in appearance and characteristics appeared on the trunk and buttocks, persisting for two days. The throat at this time showed a small, oval, punched out ulcer on the left side of the palate.

There was a persistent leucopenia (7,000 to 8,600) with a lymphocyte count from 35 per cent to 50 per cent.

Agglutination tests were negative for B typhosus, B paratyphosus, A and B paratyphosus B, and feces cultures showed no pathogens.

From the fourteenth to the sixteenth day there was increasing difficulty in swallowing, and at this time signs of meningeal irritation, rigidity of the neck and body, became increasingly prominent, a true opisthotonus occurring on the seventeenth day, with, however, alternating periods of relaxation.

During this period, tremors, hyperesthesia, internal strabismus, and incomplete ptosis of the left eyelid were all noted. Lumbar puncture, however, showed 38 cells per c mm as the only abnormality.

At this time a consultant made a diagnosis of disseminated tuberculosis with early meningeal involvement. There was, however, no history of exposure and tuberculin tests were negative.

The true diagnosis was revealed by a blood culture taken on the sixteenth day which showed numerous colonies of a gram negative bacillus later identified as *E. bentolensis*. Later in the illness (twentieth day) numerous small pustules appeared on the body from which *Staphylococcus aureus* was recovered. After a stormy tussle with the furunculosis the patient made a rapid and complete recovery.

The striking feature of the case was the similarity in many respects of the clinical picture to that seen in typhoid fever.

# THE NATURE OF THE DEPRESSOR SUBSTANCE IN HEPATIC EXTRACT\*

BY ALFRED GOERNER M.D. AND FRANK L. HALEY, M.D., BROOKLYN, N. Y.

SEVERAL years ago our attention was attracted to Major's work<sup>1</sup> on liver depressant substance. With the idea of ascertaining something about the chemical nature of this material, we undertook the following work. A liver extract was prepared as follows. Fresh lamb's liver was passed through a meat chopper and then ground still further with fine sea sand. To this mixture was added physiologic salt solution and toluol as a preservative. The mass was allowed to stand at room temperature for twenty-four hours, and then strained and mixed with an equal volume of 95 per cent alcohol. This alcoholic mixture was filtered through paper after forty-eight hours. To the clear yellow filtrate enough alcohol was added to give a concentration of 75 per cent. After one-half hour the mixture was filtered, giving a clear yellow filtrate. More alcohol was added until a concentration of 80 per cent was obtained. A precipitate formed in the 80 per cent alcoholic extract which was removed by filtration. Some of this precipitate was dissolved in distilled water and on testing gave a slight biuret reaction. The 80 per cent alcoholic filtrate was then made up to an alcoholic concentration of 90 per cent and the precipitate removed by filtration. The following tests were made on this precipitate:

Hopkins Cole	0	Fehling's	+
Molisch	+	Benedict Fehling	+
Biuret	0	Chlorides	0
Millon's	0	Uric acid	0
Xanthoproteic	0	Urea	0
Phosphorus	++++	Bile acids	0
Sulphur	+	Bile pigments	0
Cholesterol	0	Choline	0

Some of the 90 per cent filtrate was evaporated to dryness. A brown glis-tening residue was obtained which was easily soluble in water and gave the following tests:

Laessaigne's method for nitrogen	0
Iron	0
Biuret	0
Millon's	0
Xanthoproteic	0
Hopkins Cole	0

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It was distinctly positive for Molisch, reduced Fehling's solution well before and after hydrolysis but apparently to a greater extent after hydrolysis. It chattered with concentrated sulphuric acid on heating.

The 90 per cent alcoholic precipitate was dissolved in water and enough 95 per cent alcohol added to give a concentration of 50 per cent. No precipitate or turbidity was noticed. An equal volume of acetone was added and the mixture allowed to stand overnight at room temperature. The next morning the supernatant liquid was clear and the bottom of the flask showed a deposit of fine crystals. Some of these were dried at room temperature and then dissolved in 5 c.c. distilled water. One c.c. of this solution injected intravenously into a cat reduced the normal pressure to zero. The animal died within two minutes after being injected. There were no convulsions, just two or three deep respirations.

This precipitate gave a negative test for bile salts but extremely good fusion and ammonium molybdate tests for phosphorus. Quantitative determination of some of the dried crystals showed 51.35 per cent of  $P_2O_5$ . Some of the purified crystals showed 98.8 per cent  $KH_2PO_4$ .

A second batch of liver extract was then prepared and the acetone precipitate consisting of fine crystals was dried and used in our subsequent experiments. These crystals before purification showed  $KH_2PO_4$  content of 39.17 per cent. Of this precipitate 0.571 gm. corresponding to 0.224 gm. of  $KH_2PO_4$  was dissolved in 1 c.c. water and a cat weighing 3640 gm. was injected with 0.25 c.c. of this solution. The pressure dropped from a normal of 174 mm. to 92 mm. A second injection of 0.25 c.c. was given after the pressure returned to normal and once more the pressure dropped from 174 to 107 mm. A second cat weighing 2050 gm. was then injected with 0.25 c.c. of a solution of pure  $KH_2PO_4$  (0.2 gm. in 1 c.c. of water). The normal pressure dropped from 172 to 92 mm. When the pressure had returned to normal, a second injection of 0.5 c.c. was given and the pressure dropped from 172 to 86 mm.

At this stage of our work it was considered advisable to analyze several liver depressant substances recently placed upon the market by well-known firms designated as "A" and "B." Firm "A" preparation (a solution) showed the presence of approximately 1 per cent  $KH_2PO_4$ . Firm "B" preparation gave 2.3 per cent  $KH_2PO_4$ . Three solutions representing "A," "B," and our own liver extract were made up to an equivalent strength on a basis of  $KH_2PO_4$  content and all three solutions dialyzed through collodion sacks until we failed to get tests for phosphates in the diffusates. The contents of each sack were then concentrated on a water-bath to an equivalent amount and blood pressure determinations were run on cats with these solutions. None of the solutions showed any depressing effect on the normal pressure.

The respective diffusates were then concentrated on a water-bath to dryness, and the residue dissolved in distilled water. Blood pressure determinations were then made on animals with these solutions and marked depression noted, as shown in our table.

We then used a solution of  $NaH_2PO_4$  equivalent in strength to the potassium phosphate which we had been using in our previous experiments. We found that this solution did not depress the normal blood pressure of cats. It

occurred to us that the potassium ion might be responsible for the drop in pressure. Accordingly solutions of KCl equivalent in strength to the potassium in our phosphate solutions were tried out with a marked reduction in pressure. In the following table will be found a summary of our most important data. Some experiments were run on animals whose pressure had been raised with guanidine solutions previously administered, but the outcome of these was essentially the same. We also observed as did Major<sup>1</sup> that comparatively large doses were necessary to lower the normal pressure of the animals, this being true of our liver extract with a known potassium phosphate content as well as in the case of the pure potassium salt solutions.

GRAPH NO	BLOOD PRESSURE AT START MM	DEPRESSION MAXIMUM IN MM	SOLUTION USED IN CC	REMARKS
21	106	106	1 cc our own liver extract	Death of animal in 2 min
22	196	12	0.5 cc our extract	
23	196	146	1 cc our extract	Death of animal soon after
34	174	82	0.25 cc our extract	Pressure returned to normal
35	174	67	0.25 cc our extract	Animal survived
36	172	80	0.25 cc mono potass phosphate	Pressure returned to normal
37	172	86	0.5 cc as in No 36	Pressure returned to normal
45	181	0	1 cc of Firm 'A' concentrate in sack after dialysis	
46	181	0	1 cc of Firm 'B' concentrate in sack after dialysis	
47	181	0	1 cc of our concentrate in sack	
51	168	32	0.5 cc "A" diffusate concentrate	Returned to normal
52	168	25	0.5 cc "B" diffusate concentrate	Returned to normal
53	168	36	0.5 cc our diffusate concentrate	Returned to normal
62	185	0	0.5 cc mono sodium phosphate	
63	185	0	1 cc mono sodium phosphate	
66	178	21	0.5 cc KCl	Returned to normal
67	178	32	1 cc KCl	Returned to normal

Animals of nearly the same weight were used in the experiments

#### CONCLUSION

As a result of our investigations on liver extract we believe that the depressor substance is monopotassium dihydrogen phosphate with possibly traces of other potassium salts aiding this action. It would also seem that the potassium ion is the active depressor agent since other phosphates fail to cause depression and other potassium salts react positively.

#### REFERENCES

- 1 Major, R. H. The Action of Hepatic Extract in Hypertension, J A M A 87 311 July 31, 1926
- 2 Major R. H. and Stephenson W. The Effect of Methyl Guanidine on the Blood Pressure Bull Johns Hopkins Hosp 35 140, May, 1924

# THE ALLEGED SYNERGISM OF MAGNESIUM SULPHATE AND MORPHINE

## PRESENT STATUS OF THE CONTROVERSY

BY HARRY BECKMAN,\* M D, MILWAUKEE, WIS

G WATHMEY'S recent discussion of the alleged synergism of magnesium sulphate and morphine<sup>1</sup> provokes this present reply. I shall employ the following topic heads: (1) Definition of Synergism, (2) Prediction of Synergism, (3) Animal Experimentation, (4) Clinical Studies, (5) Summary.

### DEFINITION OF SYNERGISM

Synergism is the augmentation of the action of one drug by another, the latter drug to be placed in contact with the organism before or during the application of the drug whose action is augmented. Such a definition has formed the basis of all the pharmacologic investigations of synergism, but Gwathmey, who has been writing on the subject for a good many years, has never accepted it. In his recent paper, just cited, he writes: "By synergism (a Greek derivative meaning 'working together with') is meant the reciprocal augmentation of the action of one drug by that of another." This is exactly what synergism is not. Augmentation takes place, of course, but not necessarily reciprocal augmentation. In my own laboratory, as doubtless in that of any class A medical school, the students each year delight in the spectacular demonstration that magnesium sulphate will synergize urethan, but that the reverse is not true, i.e., urethan will not synergize magnesium sulphate.

### PREDICTION OF SYNERGISM

Gwathmey neither in his original pronouncements on the subject of the alleged synergism of magnesium sulphate and morphine<sup>2</sup> nor in the later applications of this synergism which he has proposed<sup>3</sup> has brought forth any animal or human experimental work that proves or even strongly indicates the admissibility of the contention. Therefore, granting that he has at all times remembered that addition and synergism must not be confused the one with the other, it can only be assumed that he still believes this particular synergism must take place because of the essential nature of the substances, i.e., the one (magnesium sulphate) an indifferent narcotic, the other (morphine) an alkaloidal narcotic. That such predictions cannot be made, and that only actual experimental studies can be accepted as having any scientific value, was shown in my review<sup>4</sup> of the literature that appeared during the controversy waged on this point in 1910 to 1915 by Burgi<sup>5</sup> and his opponents. Space does not permit re-presentation of this mass of conclusive evidence.

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## ANIMAL EXPERIMENTATION

I shall discuss here the work of the three investigators who have studied this subject in the laboratory. Issekutz, Gwathmey and Beckman. The studies of Issekutz and myself will be presented only in summary, because of their exhaustive nature, Gwathmey's contribution can easily be presented *in toto*.

(1) *Issekutz*—In 1915 Issekutz<sup>5</sup> studied the effects of magnesium sulphate on rabbits in the hope of finding some way of increasing its safe range of sedation in the treatment of tetanus. He combined it with other drugs and presented thoroughly convincing experimental evidence of the following. When magnesium sulphate is combined with scopolamine there is a true potentiation of the action, when it is combined with chloral hydrate, paraldehyde, hedonal, or phenobarbital (luminal) the sum total effect is only addition, and there can be no question of synergism, and when it is combined with morphine, not only does potentiation (synergism) not take place, but also the combination is more toxic than either the magnesium sulphate or the morphine alone.

In 1921 when Gwathmey made his original pronouncement regarding the synergism of magnesium sulphate and morphine,<sup>7</sup> he seems to have been unfamiliar with this work of Issekutz, for he wrote "A search of the literature does not give a single reference to the use of magnesium sulphate with morphine for analgesic purposes." Nor has he yet taken cognizance of the investigations of the Austrian pharmacologist though I brought the work to his attention in 1925<sup>4</sup> and Hatcher reminded him of it again in 1927.<sup>8</sup>

(2) *Gwathmey*—Gwathmey states that the combination of magnesium sulphate and morphine was first suggested to him by Pellini, and from the work they performed together he offers the following as his sole experimental evidence of the occurrence of the alleged synergism:

"A sufficient number of animal experiments was conducted to prove that one eighth gram of morphine in 2 c.c. of a 25 per cent solution of chemically pure and sterilized magnesium sulphate given hypodermically and repeated twice at half hour intervals analgized an animal sufficiently for the full force of an artery clamp to be placed anywhere on the skin without being noticed by the subject. The controlled animal with the same dosage of morphine given in plain water hypodermically, was not analgized to anything like this degree."

That is all. The size of the animals used is not mentioned, nor is the number of experiments performed stated. Also to accept the mere statement that dogs given morphine alone were "not analgized to anything like this degree" as one having any scientific value whatever is impossible, especially as it is well known that dogs become markedly insensitive to pinching on even very small doses of morphine though they will at the same time resist cutting and also show signs of heightened activity of the more superficial reflexes, such as to light and sound. Animal experiments of this sort are utterly without value.

(3) *Beckman*—Several years ago, while studying ether-oil anesthesia in the laboratory, I undertook to reduce the amount of ether employed by availing myself of the depression induced by the parenteral introduction of mag-



nesium sulphate Finding very early, however, that the ether was already employed at its minimum effective dose or nearly so, I then directed my attention toward a reduction of the morphine Here again I employed magnesium sulphate, this time for the synergistic effect that it is alleged to have upon morphine Early failure to obtain evidence of the occurrence of this synergism, plus the paucity of literature upon the subject led to a thorough investigation The colonic anesthesia experimental arrangement was well suited to the purpose, an arrangement in which the coincident employment of morphine and ether-oil, in properly adjusted doses, always gave surgical anesthesia I therefore addressed myself to the task of determining the part played by each factor in bringing about this result, in order that I might definitely fix the amount of sedation accomplished by the morphine This was necessary if I wished to observe any increase of sedation which might be due to the addition of magnesium sulphate, since *we are unable to describe as synergism the effect induced by the addition of one drug to another unless we have first studied the effect of the original drug alone* As a result of these initial experiments the amount of sedation always accomplished by the morphine was very definitely determined, as shown in the following brief summary

(1) When 1 ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 20 pounds (9 kg) body weight was introduced into the colon of dogs, one hour after the subcutaneous injection of 0.01 gm morphine sulphate per kilo body weight, safe and satisfactory surgical anesthesia was induced and maintained for a little more than one hour (the criteria for this state of anesthesia, employed in all cases, were described in the reports)

(2) When less than the above stated doses of either ether oil or morphine sulphate or less than the above-stated time interval was employed, anesthesia was not induced even though the other factors were at their effective points

(3) The effective dose of morphine was then fractionated, and it was shown that only beyond 0.0075 gm per kilo did light anesthesia begin to appear, and that only when the full dose of 0.01 gm per kilo was employed did the resultant anesthesia satisfy the criteria for the state employed throughout

Thus it was shown that in a given experimental arrangement a given amount of morphine always accomplished a certain amount of sedation, namely, that amount which was always sufficient to enable the ether to overcome the animal This was looked upon as establishing the definite sedative power of the morphine under these conditions and was therefore utilized as the basis of subsequent attempts to raise this power by the addition of magnesium sulphate The drugs were employed as follows in three series of experiments (a) Effective dose of morphine plus magnesium sulphate, effective time interval less than effective dose of ether oil (b) Less than effective dose of morphine plus magnesium sulphate, effective time interval, effective dose of ether oil The dose of morphine was fractionated here in exactly the same way as in the initial experiments in which the effective dose had been determined (c) Effective dose of morphine plus magnesium sulphate, but the magnesium sulphate given one-half hour before the morphine, effective time interval, effective dose of ether oil

In no instance was I able to observe an increase in the sedative power of the morphine due to the employment of magnesium sulphate, i.e., in all cases anesthesia was induced only when all factors were adjusted as had been shown to be necessary in the initial studies in which magnesium sulphate had not been employed. Also a peculiar reaction manifested by the dogs enabled me quite unexpectedly to note that not only was the sedative power of the morphine not raised by the magnesium sulphate but also its analgesic power was likewise unaffected. In the course of the studies 113 experiments were performed, employing 51 animals. Full accounts of the work were published<sup>9</sup> in due detail, together with the conclusion that the alleged synergism does not occur in the dog.

As the result of Gwathmey's attack upon these findings<sup>10</sup> I sought to clarify the matter by pointing out<sup>11</sup> that issue could be taken with the work only (a) when it could be shown that exactly similar investigations had been made with entirely different results or (b) when evidence is offered that the subject has been investigated upon animals using an entirely different experimental arrangement, with interpretations at variance with those I made upon my own experimental arrangement. I further stated that, since the alleged synergism of magnesium sulphate and morphine was alone being considered, discussion of the relationship of magnesium sulphate with ether and procaine had nothing to do with the subject and, indeed, served only to confuse the studies.

Gwathmey's reply to this is as follows<sup>1</sup>. Beckman further states that issue can be taken with his work only when 'it can be shown that exactly similar investigations have been made with entirely different results'. I refuse to do this as his experiments do not disprove the prolonged effect of morphine when magnesium sulphate is used instead of plain water. Further animal experiments are not necessary, as any physician can prove the synergism of magnesium sulphate with morphine by alternating the synergist with water.

Beckman states that the synergism of magnesium sulphate with ether and procaine has no bearing on the subject. With this I do not agree. Magnesium sulphate does potentiate the value of many drugs and in different ways. It is reasonable, therefore, to see that since it potentiates other drugs, all such experiments have a bearing on the synergism of the magnesium ion with morphine. His present position, then, so far as I am able to understand it, might be stated as follows: (a) He now throws out all of my work for the reason that it does not deal with the length of time that morphine is assumed to maintain contact with the tissues when it is given with magnesium sulphate, despite the fact that in his own experimental work (previously described in full in this present paper) he concerned himself solely and entirely with the alleged ability of magnesium sulphate to raise the immediate analgesic power of the morphine, and also despite the fact that his previous objections to my work have been put upon the ground that there was a disproportion between my magnesium sulphate and morphine doses. (b) He says that animal experimentation is not necessary anyway, since any practitioner can easily and simply determine whether the effect produced is one of addition or of synergism by injecting a patient with mor

phine in water and then again in magnesium sulphate solution (c) He states that this synergism can be assumed to take place because magnesium sulphate synergizes with a number of other substances

#### CLINICAL STUDIES

I have several times stated my reasons for believing that none of the published clinical studies of this subject indicate a synergistic effect of magnesium sulphate upon morphine, a position in which I am supported by Hatcher,<sup>8</sup> who in 1927 reviewed the matter for the Council on Pharmacy and Chemistry of the American Medical Association. At this time, therefore, I shall only consider Gwathmey's own clinical proof, of which he has written<sup>10</sup> "Satisfactory evidence is submitted to show that synergism between magnesium sulphate and morphine *does* occur in man." This evidence is submitted in the form of three tables. Tables II and III comprise lists of cases in which the time before postoperative sedative was shown to be longer when morphine plus magnesium sulphate was used preoperatively than when morphine was used alone. This is certainly no evidence of synergism. Both experimental and clinical literature offer abundant proof that magnesium sulphate is a powerful sedative, which evidence I believe no one has ever sought to question. But nothing is offered in connection with these two tables to show that the increase in sedation was not due to the simple additive effect of the two drugs. Gwathmey's third table, which I present here as Table I, is described by him as "the first classic case in which the potentiation of morphine was established beyond all reasonable doubt."

Having had it pointed out to him<sup>11</sup> that the second longest period of relief

TABLE I

CASE ILLUSTRATING DIFFERENCE BETWEEN MAGNESIUM SULPHATE AND PLAIN WATER WHEN USED WITH MORPHINE (GWATHMEY)

BADLY LACERATED WOUND OF LEG, GAS INFECTION, OPENED WIDE DECEMBER 1, GASTROCNEMIUS SEVERED				Duration
December				Hours
2	1 15 A M	Morphine, $\frac{1}{8}$ , MgSO <sub>4</sub> , 2 cc, 25 per cent, quiet until 5 A M		5 $\frac{3}{4}$
	6 15 A M	Morphine, $\frac{1}{12}$ , MgSO <sub>4</sub> , 2 cc, 25 per cent, no pain all day The magnesium was now discontinued. Morphine alone used		18
3	1 30 P M	Morphine, $\frac{1}{8}$ , pain in two hours		2
	3 25 P M	Morphine, $\frac{1}{6}$ , pain at 6 40 P M The magnesium was again added to the morphine		3 $\frac{1}{4}$
	6 55 P M	Morphine, $\frac{1}{6}$ , MgSO <sub>4</sub> , 25 per cent, 2 cc, no pain till next day		23
4	5 00 P M	Morphine, $\frac{1}{10}$ , MgSO <sub>4</sub> , 2 cc, 25 per cent, no pain till next A M		17
5	10 15 A M	Morphine, $\frac{1}{10}$ , MgSO <sub>4</sub> , 3 cc, 25 per cent, quiet all day		10
	8 15 P M	MgSO <sub>4</sub> , 3 cc, very good night		19
6	3 50 P M	MgSO <sub>4</sub> , 3 cc, 25 per cent, severe pain after three hours		3
	6 15 P M	Morphine, $\frac{1}{8}$ , MgSO <sub>4</sub> , 2 cc, 25 per cent, jerking of leg in two hours		2
	9 15 P M	Morphine, $\frac{1}{8}$ , MgSO <sub>4</sub> , 2 cc, 25 per cent, quiet night		19
The patient required one hypodermic of morphine, $\frac{1}{10}$ to $\frac{1}{8}$ , and MgSO <sub>4</sub> , 2 cc, 25 per cent, each day after this until Dec 13. Codeine and morphine alone did not give relief				

in this case was experienced in one of the two instances only in which magnesium sulphate was used alone Gwathmey replies 'Pain was seemingly suppressed for nineteen hours in the same patient by 3 c.c. of magnesium sulphate alone, but this was subsequent to the previous injection of morphine and magnesium sulphate, and one has a right to conclude that a hang over resulted. This seems to be proved by the fact that with the next hypodermic of magnesium sulphate alone 3 c.c. of a 25 per cent solution, there was severe pain after three hours as by this time all the morphine had disappeared from the system.' That is to say, the particular effect that followed the 8 15 P.M. injection of magnesium sulphate alone on Dec. 5 is to be attributed to 'hang over' from the previous injection of morphine plus magnesium sulphate, but the good effects that followed the 6 15 A.M. injection on Dec. 2, the 5 P.M. injection on Dec. 4, the 10 15 A.M. injection on Dec. 5 and the 9 15 P.M. injection on Dec. 6 are not to be attributed to hang over, though they also followed previous injections of the two drugs in combination. These particular effects are to be looked upon as due to synergism.

Furthermore, there is direct evidence in this table that in at least three instances the good results obtained by the combined use of magnesium sulphate and morphine are directly attributable to addition and to addition only—the contention is supported by Gwathmey's own testimony. He has said 'Neither I nor any one else at any time has hinted that smaller doses of morphine could be used 'to overcome the pain' in the presence of magnesium sulphate in any amount. What I said is that it seems to act mechanically with morphine, holding it in contact with the tissues longer than morphine can maintain such contact alone.' Hence as I have distinctly stated, the same amount of morphine must be used with magnesium sulphate as with sterile water. In other words one eighth grain of morphine is not converted into one sixth or one sixth into one fourth or one fourth into one half.' Now I understand this statement to mean—and it is the only possible meaning that it can have—that if an inadequate dose of morphine is given to a patient i.e., a dose much smaller than would be given for the relief of severe pain, then no matter how much magnesium sulphate is given with this inadequate dose the synergistic effect of the magnesium sulphate upon the morphine will not be exhibited, for the reason that the only effect that magnesium sulphate has upon morphine is to hold it for a long time in contact with the tissues but not in any way to raise either its sedative or analgesic power. Therefore, in the case under consideration, synergism most certainly could not have occurred at the 6 15 A.M. injection on Dec. 2, nor at the 5 P.M. injection on Dec. 4, nor at the 10 15 A.M. injection on Dec. 5, for on all these occasions an entirely inadequate dose of morphine was used. Yet excellent results followed each of these three injections, results which could only have been due to an adequate dose of magnesium sulphate plus a small dose of morphine—that is to say, an additive effect solely and entirely.

#### SUMMARY

1. Gwathmey does not define synergism in a way that will satisfy any pharmacologist anywhere in the world.

2 Synergism cannot be predicted from the nature of the substances used together, nor can it be presupposed to occur because of analogous synergisms, both which propositions Gwathmey refuses to accept

3 Issekutz showed that the alleged synergism does not occur in rabbits, and Beckman that it does not occur in dogs Gwathmey's own experimental work has been shown to be entirely inadequate, but he refuses to perform any experiments in the attempt to overthrow the findings of Issekutz and Beckman, instead, he states that animal experimentation is not necessary, and that any physician so minded can easily prove this synergism for himself

4 All the clinical work that has been cited by Gwathmey and others has been shown in the reviews of Hatcher and Beckman to prove nothing with regard to the occurrence of this synergism in man

5 In the laboratory the alleged synergism can be proved to occur only when it can be shown that (a) exactly similar investigations to those of Beckman have been made with entirely different results, or (b) an equally adequate but different experimental arrangement from that used by Beckman has been used with entirely different results

6 In the clinic the alleged synergism can be proved to occur only when it can be shown that the good result following the coincident use of the two drugs is not due to simple addition of their separate effects

#### REFERENCES

- 1 Gwathmey, J T J A M A 91 1774, 1928
- 2 Gwathmey, J T J A M A 77 421, 1921
- 3 Gwathmey, J T, Donovan, E P, and Cowan, L R Am J Obst & Gynec 6 456, 1923 Gwathmey, J T, McKenzie, R A, and Hudson, F J Am J Obst & Gynec 8 154, 1924
- 4 Beckman, H J A M A 85 332, 1925
- 5 Buigi Deutsche med Wchnschr 1910, Nos 1 and 2, Ztschr f exper Path u Therap 8 523, 1911 Breslauer and Waker Ztschr f allg Physiol 13 282, 1912 Issekutz Arch f ges Physiol 145 415, 1910, ibid 151 456, 1913 Straub Biochem Ztschr 41 1912 Ceasar Biochem Ztschr 42 316, 1912 Overton Studien uber die Narkose, 1901, p 166 Lemonsof Ztschr f exper Path u Therap 8 566, 1911
- 6 Issekutz, B Therap Monatschr 29 379, 1915
- 7 Gwathmey, J T J A M A 76 222, 1921
- 8 Hatcher, R A J A M A 89 2189, 1927
- 9 Beckman, H J LAB & CLIN MED 10 189, 1924, J A M A 85 332, 1925
- 10 Gwathmey, J T J A M A 85 1482, 1925
- 11 Beckman, H Am J Obst & Gynec 15 72, 1928

# COMPARATIVE INHIBITING EFFECT OF GENTIAN VIOLET AND MERCUROCHROME ON THE GROWTH OF CERTAIN FUNGI\*

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EARLIER studies<sup>1</sup> on the comparative inhibiting effect of gentian violet and mercurochrome on bacterial growth in vitro elicited some very striking results. It is well established that basic dyes are increasingly effective as inhibiting agents as the  $P_H$  of the medium is increased, and that acid dyes show an opposite tendency, i. e., at higher  $P_H$  values their effectiveness is less. Gentian violet is a basic dye, while the dye from which mercurochrome is prepared is an acid dye. Thus it was found that at blood  $P_H$  gentian violet was exerting a maximal effect while mercurochrome was less effective than in more acid solutions. Table I will illustrate the marked differences in the limiting effective dilutions of the two at  $P_H$  7.4. The results are given for growth after a ninety six hour incubation period.

TABLE I

ORGANISM	LIMITING EFFECTIVE DILUTION		RATIO
	Gentian Violet	Mercurochrome	
B. coli	50 000	1 500	33
B. dysenteriae (Shiga)	200 000	5 000	40
B. diphtheriae	400 000	5 000	80
Staphylococcus aureus	1 000 000	5 000	200

Later it was shown that certain fungi were markedly sensitive to basic dyes, and the question arose whether a similar difference in sensitivity to the above two inhibiting agents would be exhibited by the more complex fungi. In order to determine this, a Blastomyces, two Oidia and three Hypomyceses were cultured in media containing gentian violet and mercurochrome. The following cultures, used in the experiments described below, were obtained from Dr. M. P. Moon:

## *Blastomyces*

*Oidium mycosum* (isolated from an infected hand)

*Oidium albicans* (isolated from an infected tongue)

*Trichophyton rosaceum*

*Trichophyton granulosum*

*Achorion schoenleinii* (Am. Type Culture Collection, H 641)

They were first subcultured in 1 per cent sugar broth and then transferred to nutrient bouillon and agar slants. Since practically all of the cultures used appear to grow readily in a slightly acid nutrient bouillon after repeated transfers and since they seem to grow readily through only a limited  $P_H$  range,

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results are given for growth at only one  $P_{11}$ , which was near the optimum in most cases

Nutrient bouillon containing varying amounts of gentian violet or mercurochrome and adjusted in reaction by a phosphate mixture in an amount so that the total phosphate concentration was  $M/15$  was inoculated with a loopful of a bouillon culture. In case the fungus grew in mass it was shaken and macerated with a steel needle. Since it was unusually difficult to insure anything like equal amounts of inoculum, an unusually large number of series had to be run and the results homologized, thus differing from the results obtained in studying bacteria, where not only complete series but also gradations in growth within a single series were constantly checked.

Tables II to VII give the results. Data are also included showing the limiting inhibiting dilutions of acid fuchsin. This permits one, by comparison, to note the added inhibiting effect caused by introducing mercury into the acid dye molecule as was done in the case of mercurochrome. The results are given for growth (+) after incubation periods of two four and eight days respectively.

TABLE II  
BLASTOMYCES

GENTIAN VIOLET		$P_{11}$		MERCUROCHROME		$P_{11}$		ACID FUCHSIN		$P_{11}$
DILUTION		64	68	DILUTION		64	68	DILUTION		64
1	500,000	-	-	1	5,000	-	-	1	25	-
		-	-			-	-			-
		-	-			-	-			-
1	1,000,000	-	-	1	10,000	-	-	1	50	+
		+	-			+	+			+
		+	+			+	+			+
1	1,500,000	-	-	1	20,000	+	+	1	100	+
		+	+			+	+			+
		+	+			+	+			+

TABLE III  
TRICHOMYCTON ROSACEUM

GENTIAN VIOLET		$P_{11}$		MERCUROCHROME		$P_{11}$		ACID FUCHSIN		$P_{11}$
DILUTION		64	68	DILUTION		64	68	DILUTION		64
1	4,000,000	-	-	1	5,000	-	-	1	25	-
		-	-			-	-			-
		-	-			-	-			-
1	8,000,000	-	-	1	10,000	-	-	1	50	-
		-	-			-	-			-?
		+	-			-	+			+
1	9,000,000	+	-	1	20,000	+	+	1	100	+
		+	+			+	+			+
		+	+			+	+			+

The data in these tables bring out the fact that the higher sensitivity to the basic dye gentian violet on the part of the fungi, compared with that of bacteria under like conditions, is not necessarily explained by assuming that fungi are by nature more sensitive to dyes in general. This is indicated by

TABLE IV  
ACHORION SCHOLENLEINII

GENTIAN VIOLET DILUTION	$P_H$		MERCURIOCHROME DILUTION	$P_H$	
	64	68		64	68
1 8 000,000	-	-	1 10 000	-	-
	-	-		-	-
	-	-		-	-
1 10 000,000	-	-	1 20 000	-	-
	-	-		-	-
	+	-?		-	-
1 20 000,000	-?	+	1 40 000	-	-
	+	+		-	-
	+	+		+	+
1 30,000,000	+	+	1 80 000	-	-
	+	+		-	-
	+	+		+	+
			1 160 000	+	+
				+	+
				+	+

TABLE V  
ODIUM MYCOSUM

GENTIAN VIOLET DILUTION	$P_H$		MERCURIOCHROME DILUTION	$P_H$		ACID FUCHSIN DILUTION	$P_H$
	64	68		64	68		64
1 10 000 000	-	-	1 20 000	-	-	1 25	+
	-	-		-	-		+
	-	-		-	-		+
1 20 000 000	-	-	1 40 000	-?	+	1 50	+
	-	-		+	+		+
	+	+		+	+		+
1 30 000 000	+	+	1 80 000	+	+	1 100	+
	+	+		+	+		+
	+	+		+	+		+

TABLE VI  
ODIUM ALBICANS

GENTIAN VIOLET DILUTION	$P_H$		MERCURIOCHROME DILUTION	$P_H$		ACID FUCHSIN DILUTION	$P_H$
	64	68		64	68		64
1 750 000	-	-	1 10 000	-	-	1 25	-
	-	-		-	-		-
	-	-		-	-		-
1 1 000 000	+	-	1 20 000	-	-	1 50	+
	+	+		-	-		+
	+	+		+	+		-
1 1 500 000	+	+	1 40 000	-	-	1 100	+
	+	+		-	-		+
	+	+		+	+		+
			1 80 000	-	+		
				-	+		
				+	+		
			1 160,000	+	+		
				+	+		
				+	+		



comparative data contained in Table VIII, which shows that there is no corresponding increase in sensitivity toward the acid dye acid fuchsin. Indeed, if anything, there seems to be a decrease in sensitivity toward this latter dye. Where the acid dye contains mercury, as in the case of mercurochrome, fungi seem somewhat more sensitive to it than are bacteria, and this seems an effect due to the mercury.

TABLE VII  
TRICHOPHYTON GRANULOSUM

GENTIAN VIOLET		$P_H$		MERCUROCHROME		$P_H$		ACID FUCHSIN		$P_H$	
DILUTION		64	68	DILUTION		64	68	DILUTION		64	
1	250,000	-	-	1	5,000	-	-	1	25	+	
		-	-			-	-			+	
		-	-			-	-			+	
1	500,000	-	-	1	10,000	-	-	1	70	+	
		+	+			-	+			+	
		+	+			+	+			+	
1	750,000	+	+	1	20,000	+	+	1	100	+	
		+	+			+	+			+	
		+	+			+	+			+	

Table IX gives a resume of results. As in the case of bacteria, it is seen that for *in vitro* work the ordinary basic dye gentian violet is distinctly more effective in inhibiting fungus growth than is mercurochrome. Under the conditions obtaining in the present experiments, the ratio of effectiveness, as shown by limiting dilutions, varies with the organism from 50 to as much as 500 times.

TABLE VIII

ORGANISM	GENTIAN VIOLET DILUTION	MERCUROCHROME DILUTION	ACID FUCHSIN DILUTION
<i>Bacteria at <math>P_H</math> 6.8</i>			
<i>B. coli</i> (gram neg.)	1 20,000	1 2,500	1 75
<i>Staphylococcus aureus</i> (gram pos.)	1 2,000,000	1 10,000	-----
<i>Fungi at <math>P_H</math> 6.4</i>			
<i>Blastomyces</i> (gram neg.)	1 500,000	1 5,000	1 25
<i>Oidium mycosum</i>	1 10,000,000	1 20,000	1 <25
<i>Trichophyton rosaceum</i>	1 4,000,000	1 10,000	1 25

TABLE IX  
LIMITING DILUTIONS AT  $P_H$  6.4 FOR THE VARIOUS ORGANISMS

ORGANISM	GENTIAN VIOLET DILUTION	MERCUROCHROME DILUTION	RATIO
<i>Oidium mycosum</i>	1 10,000,000	1 20,000	500
<i>Oidium albicans</i>	1 750,000	1 10,000	75
<i>Blastomyces</i>	1 500,000	1 5,000	100
<i>Trichophyton rosaceum</i>	1 4,000,000	1 10,000	400
<i>Trichophyton granulorum</i>	1 250,000	1 5,000	50
<i>Achorion schoenleinii</i>	1 8,000,000	1 20,000	400

# REFERENCES

- 1 Stearn, E. W., and Stearn, A. E. Am. J. Pub. Health 16: 1003, 1926
- 2 Stearn, A. E., and Stearn, E. W. Univ. of Missouri Studies 3: 52, 1928

# STUDIES OF SEDIMENTATION OF ERYTHROCYTES\*

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IN A PREVIOUS article on this subject it was mentioned that although many theories had been advanced with regard to the etiologic factors in sedimentation of blood none had been generally accepted. In view of this I investigated certain cases and attempted to correlate the possible etiologic factors with the clinical data.

That the plasma contains a substance which is responsible for the increased sedimentation of the erythrocytes is the original hypothesis advanced by Fahraeus. He believed that the increased rate of sedimentation of erythrocytes was due to their increased agglutination, and that this agglutination was chiefly dependent on the properties of the plasma. He concluded that the agglutinating capacity of the plasma was due to the protein fractions, albumin, globulin and fibrinogen. He also observed that solutions of different protein fractions of the plasma differ widely in agglutinating capacity. He said "Serum albumin aggregates the corpuscles to a very little extent, strong agglutination is produced by the serum globulins. Of the serum globulins, fibrinogen is by far the most active. The strong agglutinating effect of fibrinogen is proved by the fact that the agglutination is to a high degree reduced by defibrination of the blood."

Pfeiffer<sup>13</sup> in his observations on the formation of the 'buffy coat' of the blood noted that the increased sedimentation of the corpuscles was generally caused by increased agglutination of the corpuscles. He believed that an increased fibrinogen percentage in the plasma might cause the corpuscles to congregate more rapidly and in this way further the agglutination. Certain other investigators believe that the electrical charge of the erythrocytes exerts a significant influence on sedimentation.

I early observed that by exchanging the cells and plasma of two samples of blood the cells of the blood in which sedimentation takes place slowly when transferred to the plasma of the blood in which sedimentation takes place rapidly, give practically the same result as the original cells in the latter type of blood. If the cells of this blood were transferred to the plasma of the blood that settled slowly the result was almost the same as when the original cells were in the plasma of this blood (Table I). As this phenomenon occurred quite consistently in all samples of blood obtained from patients who were in compatible groups as determined by blood typing methods, it would seem that the substance that controls the sedimentation of the erythrocytes is contained in the plasma.

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With Fåhræus' theory in mind, I carried out a number of experiments to determine if possible whether a relationship existed between the fibrinogen content of the blood expressed as fibrin, the sedimentation index and the clinical condition of the patient. All the laboratory and clinical data were tabulated and observed. In other words my purpose was to correlate any information obtained, either clinically or in the laboratory, with the sedimentation index. I thus hoped to determine the factor or factors that influence the sedimentation of erythrocytes.

The specimens were all obtained from ambulatory patients whose blood was examined as a routine. The method of collecting the specimen of blood and the technique of the sedimentation test were described in my previous article.

TABLE I

MIXTURE OF PLASMA AND CELLS FROM SLOWLY SETTLING AND RAPIDLY SETTLING BLOOD

TIME, HOURS	TUBES A*		TUBES B**		SLOWLY SETTLING BLOOD	RAPIDLY SETTLING BLOOD
0.25	0.070	0.070	0.005	0.002	0.002	0.017
0.50	0.205	0.370	0.012	0.012	0.020	0.220
1.0	0.410	0.440	0.025	0.025	0.060	0.355
2.0	0.465	0.465	0.090	0.085	0.065	0.440
3.0	0.490	0.485	0.012	0.120	0.100	0.460

\* Contain 0.5 cc of cells from slowly settling blood in 0.5 cc plasma from rapidly settling blood.

\*\* Contain 0.5 cc cells from rapidly settling blood in 0.5 cc plasma from slowly settling blood.

The method used for determining the fibrin content of the blood is one perfected by Schultz, Nicholes and Schaefer.

In the 57 cases in which fibrin determinations were made, there were fourteen men and three women with sedimentation indexes within normal limits (men 0.1 to 2.0 and women 1.1 to 3.4). In this group the average sedi-

TABLE II

NORMAL SEDIMENTATION INDEXES AND NORMAL FIBRIN VALUES IN VARIOUS CONDITIONS

CASE	DIAGNOSIS	AGE AND SEX	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	SEDIMENTATION INDEX	FIBRIN, MG PER CENT	BLOOD PERFUSION	
							SYSTOLIC	DIASTOLIC
1	Parkinson's syndrome	18M	5.02	82	2.4	266.6	129	80
2	Diabetes mellitus	52M	4.45	75	2.6	277.7	136	84
3	Normal	40M	4.81	73	1.2	169.4	116	80
4	Chronic nervous exhaustion	35M	4.60	84	1.7	193.3	140	75
5	Exophthalmic goiter	19M	4.50	77	1.1	231.1	164	70
6	Migraine	32M	4.80	81	1.3	166.6	125	75
7	Psychoneurosis	36M	4.66	78	2.0	157.7	120	78
8	Asthma	37M	4.29	72	2.0	235.5	116	76
9	Sacroiliac strain	32M	4.75	76	2.1	190.0	120	88
10	Migraine and chronic nervous exhaustion	38M	4.50	78	1.7	190.0	120	90
11	Chronic prostatitis	40M	4.85	79	1.4	108.3	115	85
12	Dementia præcox	27M	4.70	75	0.9	138.6	112	76
13	Duodenal ulcer	25M		77	1.1	195.2	108	72
14	Keloid of chest wall	30M	4.59	76	0.9	115.0	140	74
15	Normal	43F	4.80	81	3.0	245.9	110	75
16	Septic tonsils	47F		73	3.4	182.9	142	88
17	Chronic myocarditis	59F	4.38	71	3.4	266.6	155	85

mentation index for men was 1.6 and the average fibrin values were 178.3 mg for each 100 cc of blood. The average sedimentation index for women was 3.2 and the average fibrin values 231.4 mg for each 100 cc of blood. The average hemoglobin for the men with normal sedimentation index values was 77 per cent on the Dacie hemoglobinometer, and the average erythrocyte count was 4,655,000. The average hemoglobin for the women with normal sedimentation index values was 75 per cent on the Dacie hemoglobinometer and the average erythrocyte count was 4,590,000. The blood pressure of these patients was normal. None of the women in the group was menstruating.

In all of the cases tabulated in Table II the sedimentation indexes and the fibrin value were normal. Since the normal fibrin value cannot be fixed arbitrarily, I allowed some variation either above or below the average fibrin values found. The parallelism between the increase in fibrin and the increase in the sedimentation index is shown in Fig. 1.

The sedimentation index was determined on 557 specimens of blood obtained from patients as a routine during their examination and nothing was known concerning them except the name and registration number. In this

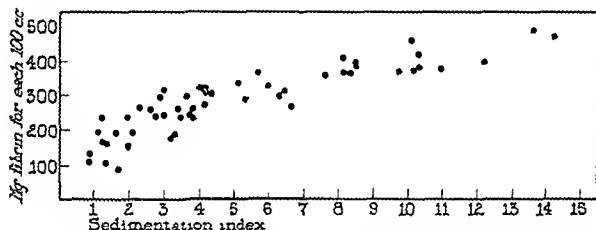


Fig. 1—The relation between the fibrin content of the blood and the sedimentation index.

group of mixed cases no attempt was made to correlate the results obtained in any one case with another. In fact the histories were not examined for several months after the fibrin and sedimentation index determinations were made.

Grum's extensive study of the fibrin content of the blood plasma in health and disease gives the normal values as maximal 360, minimal 200 and average 270 for men and maximal 380, minimal 210 and average 290 for women.

Foster in a series of 42 determinations found the average blood fibrin for normal men to be 163 mg for each 100 cc of blood and for normal women 179 mg. Variations above or below these figures may be said to be within normal limits. The average plasma fibrin for normal men was 332 mg for each 100 cc and for normal women 344 mg. Variations of 50 mg above or below these figures are probably within normal limits.

That the fibrin content of the blood is increased in a few physiologic conditions and in many pathologic conditions has been established.

#### PHYSIOLOGIC HYPERTENSION

Lewinski Klossing and Landsberg found that the plasma of a pregnant woman is 45, 100, and 20 per cent respectively richer in fibrin than that of

a nonpregnant woman. De Lee believes that the increase of fibrin is probably a conservative act of nature to prevent dangerous loss of blood during labor. He stated that the amount of fibrin may be increased by one-third.

Fahraeus found that during pregnancy the "suspension stability" of the blood is almost always greatly reduced, this, according to his theory, means an increase in the fibrin content of the blood.

Giam<sup>22</sup> observed that in the beginning of normal pregnancy there is a tendency to hyperinosis, but that it does not always exceed the upper limit of normal before the fifth or sixth month.

Foster found the average blood fibrin of pregnant women to be 273 mg for each 100 cc and the average plasma fibrin 415 mg for each 100 cc. He believes that these figures are probably applicable only for the later months of pregnancy.

Chandler, in a series of duplicate determinations, noted a fairly consistent increase in the fibrin values, particularly in the later months of pregnancy.

Many investigators have studied the changes produced in the blood picture by menstruation, but only a few have investigated the fibrin content of the blood in relation to menstruation.

The fibrin content of the blood has been found to be increased in the newborn and in the aged.

#### PATHOLOGIC HYPERINOSIS

Fibrin values in pathologic conditions have been subjected to extensive investigation. Although various methods have been devised and used in the determination of fibrin, the results have been fairly constant.

According to Halliburton, Laeksehewitz, Beiggin and Dochez, the fibrin has been found to be increased in many infectious diseases, such as pneumonia, pulmonary tuberculosis, pleuritis, peritonitis, acute rheumatic fever, erysipelas and septicemia.

Pfeiffer<sup>43</sup> was the first to report an extensive series of cases in which the content of fibrin of the blood in disease had been estimated. In general he found an increase of the fibrin of the blood in the presence of diseases which are accompanied by leucocytosis (pneumonia, scarlet fever, erysipelas, and acute rheumatic fever), but in leukemia the fibrin content of the blood was normal. In diseases without leucocytosis (typhoid fever, nephritis and malaria) the content of fibrin was normal.

Kollmann found an increase in the fibrin of the blood of patients suffering from eclampsia, this was not substantiated by Lewinski.

Because of the poor coagulation of leucemic blood it would naturally be supposed that fibrin is deficient. Some investigators have found deficiency in fibrin and others have found an increase. Pfeiffer<sup>44</sup> found the amount of fibrin to be nearly normal. Erben found a normal amount of fibrin in the blood in three cases of myelogenous leukemia and in one case of lymphatic leukemia. According to present knowledge, the failure of leucemic blood to clot cannot be attributed to the decrease in fibrin.

In chlorotic blood, Erben's analysis showed that the total amount of pro-

ten is decreased, chiefly because of the deficiency of hemoglobin the relation of serum albumin is unchanged, and the proportion of fibrin is increased

Following severe hemorrhage Drinker and Hurwitz noted a decrease in the fibrin, while in aplastic anemia<sup>5</sup> the amount was normal. In pernicious anemia, according to Wells,<sup>54</sup> the fibrin is decreased in total amount, but is relatively normal as compared with the total proteins. In regard to hemophilia Wells<sup>5</sup> stated "Since bleeding is normally stopped principally by coagulation a deficiency in fibrin or its antecedents might be expected, but most studies on this point have shown a normal amount of fibrinogen in the blood of hemophiliacs."

The most extensive series of fibrin determinations in health and disease published thus far is that of Gram.<sup>2</sup> His figures for the normal fibrin values have been quoted previously. He found the fibrin content of the plasma to be increased in almost all infectious diseases, in carcinoma, nephritis, pregnancy, polyarthritis and after injections of sterile milk. A low percentage of fibrin in plasma is found only in certain types of severe degeneration of the liver. Gram found fibrin deficiency in four of a series of 23 cases of pernicious anemia, in the others the fibrin values were within normal limits. In simple anemia the percentage of fibrin in plasma was normal, although the percentage for each 100 cc of blood was high. In polycythemia vera the percentage of fibrin in plasma also varied within normal limits while the percentage of fibrin in blood was low. From the results observed in anemia and polycythemia vera he concluded that the percentage of fibrin in plasma is the value which remains fairly constant when the cell volume is altered. In leucemia, especially in the myeloid type, and in hemophilia he found a slight absolute increase of the fibrin percentage in the plasma.

In a recent study Foster, in a much smaller series of cases substantiated Gram's observations.

#### PREGNANCY

Fahraeus considered rapid sedimentation of erythrocytes as one of the early signs of pregnancy. Sakae and Tsutsumi also noticed that the rate of sedimentation in pregnant women was far greater than that of normal blood and that the rate increased as pregnancy advanced. They attributed this to two factors: that the ratio of the blood volume to the erythrocytes in the blood of pregnant women is greater, and that the corpuscles are more easily agglutinated, due to an increase in fibrinogen or globulin or to a decrease in the albumin content of the blood. Gram<sup>21</sup> called attention to the early increase in the sedimentation rate of pregnant women. He believes that the increase in sedimentation in the early months of pregnancy is due to a drop in the percentage of cell volume but in the later months the increased sedimentation rate is carried on by the increase of fibrin. Hirst and Long believe that the sedimentation test is worthless in the diagnosis of early pregnancy. This opinion is also held by Baird and Reis who examined 100 women and at no time before the twentieth week was the sedimentation time low enough to be distinctive or diagnostic. Falta after observing the sedimentation reaction in approximately 1000 cases including patients at various periods of pregnancy

concluded that the rapidity of sedimentation is constant in pregnancy after the fourth month

That there is an increase in the fibrin content of the blood as well as an increase in the rate of sedimentation of the erythrocytes of pregnant women seems to be firmly established

None of my series of 57 specimens of blood in which the fibrin content and sedimentation index were determined was taken from a pregnant woman. In four of the 500 cases in which only the sedimentation indexes were determined, the maximal and minimal sedimentation index was 102 and 68 respectively

The sedimentation test as an aid in the diagnosis of pregnancy is of little value in the early months of pregnancy, when other definite signs of pregnancy are lacking. In the later months other signs are so prominent that a more delicate test is not necessary. From the work of other investigators reported in the literature, I am convinced that pregnancy is a physiologic condition in which there is a consistent increase in the sedimentation index, particularly in the later months

The consensus of opinion seems to be that the sedimentation reaction is of little value in the early diagnosis of pregnancy. This reaction increases with the advance of pregnancy but is of little use in estimating the termination of gestation

#### TUBERCULOSIS

An increase in the sedimentation of erythrocytes in cases of tuberculosis has been noted consistently

Frisch believes that the breaking down of tissue results in the liberation of enzymes which in turn play an important part in the formation of fibrin. This view is also held by Foster and Whipple, Sakamoto and Mills

Westergaard believes that the fibrinogen fraction of the plasma increases in cases of tuberculosis and that this increase is most easily estimated by means of the sedimentation reaction. In all of his cases which, judging from the clinical signs, he considered active, the extent of the sedimentation of the erythrocytes was parallel to the degree of activity

Dreyfus and Hecht considered the sedimentation reaction significant in determining the activity of a tuberculous process and a more reliable indicator of the course of the infection in a given case than the temperature curve

Morriss, in a preliminary study, observed a definite increase in the velocity of the sedimentation of erythrocytes in active pulmonary tuberculosis. He found also that in the presence of definite symptoms of activity sedimentation was within normal limits, although it was common, especially in women to find normal readings. His final conclusion was that the test had little value in the diagnosis of tuberculosis. Two years later, in association with Rubin, he substantiated his previous work by saying "The test has been found especially useful in tuberculosis as an indication of the degree of activity present"

Grafe, Levinson, Salomon and Valtis, Bochner and Wassing, Cooper and many others have found the sedimentation test to be of more or less value in the diagnosis and prognosis of tuberculosis

Since it has been demonstrated that increased sedimentation of erythrocytes is more active when the fibrin content of the blood is increased and that there is an increase in the fibrin when there is destruction of tissue especially of the lungs, it seems probable that this is the correct explanation of the increased sedimentation of erythrocytes in cases of tuberculosis.

In summarizing the literature in regard to the use of the sedimentation reaction as an aid in the diagnosis of tuberculosis the combined conclusion seems to be that the degree of sedimentation depends on and closely parallels the extent of anatomic involvement by the disease.

In the series of specimens of blood on which I made fibrin determinations none was taken in a case diagnosed as tuberculosis. The group of cases in which only the sedimentation index was made is too small to permit conclusions from the results obtained. The blood from all of these patients gave pathologic sedimentation indexes. Three of them were advanced cases of pulmonary tuberculosis with extensive anatomic involvement of the lung. The sedimentation indexes in these three cases were all very high.

Since approximately 17 000 cases of tuberculosis have been studied by this method with similar results it must be concluded that the value of the test is in the information afforded with regard to the activity and extent of anatomic involvement of the lung. In the early diagnosis of the disease the sedimentation index is of little value. It might be of some value in helping to eliminate other conditions in which it is known that sedimentation is not increased. After the diagnosis of pulmonary tuberculosis the test may be of some value in determining whether the patient is growing better or worse. If sedimentation is increased, in the absence of change in the blood count, intercurrent infection, menstruation, or increase in the blood pressure, it would seem to indicate more extensive destruction of lung tissue, with the resulting liberation of more fibrin forming enzymes. If the reverse of this process is true and the sedimentation index approaches normal limits it would indicate the possibility of the process becoming less active. In this way the test may be of prognostic value, the higher the sedimentation index the graver the prognosis.

#### ANEMIA

As previously mentioned, the percentage of fibrin of the blood in simple anemia, aplastic anemia, and polycythemia vera is almost always within normal limits. In leucemia, pseudoleucemia and hemophilia there is only slight, if any, increase. Diminution of the fibrin percentage of the plasma is usually present in pernicious anemia. Thus some other cause must be attributed to the increase of sedimentation of erythrocytes in cases of anemia, or in some of the blood dyscrasias.

Gram<sup>22</sup> found increased sedimentation of the erythrocytes in anemia that closely parallels the decrease in the blood count. Fibrin values did not increase unless there was secondary infection.

Groedel and Hubert believe that the erythrocyte itself is the most important factor influencing the sedimentation reaction. They applied the test in 304 cases and found that in almost any internal disease, and in normal subjects, the sedimentation rate may be normal or increased. In case of high hemoglobin content and a normal number of erythrocytes the rate is usually



normal, or slightly accelerated. They also noticed that increased sedimentation is more common in blood low in hemoglobin, and the hemoglobin percentage is not related directly either to the two hour or to the twenty-four-hour sedimentation value.

Westergaard<sup>57</sup> has shown, by means of a diagram with plotted curves, that the relation between the sedimentation figures after one hour and after twenty-four hours is chiefly influenced by the hemoglobin content of the blood. By means of this diagram and the sedimentation reading for one hour and twenty-four hours the hemoglobin can be estimated with an error of less than 10 per cent.

Rubin and Smith, in studying the relation between the hemoglobin, cell count, and cell volume and the erythrocyte reaction used hirudinized blood. They found, in general, that their results, as regards the hemoglobin content, agreed with those obtained by Goeddel and Hubert. The cell count and sedimentation index were also in agreement. Since the patients in the series had chronic diseases with varying degrees of secondary anemia the decrease in the cell count was accompanied by a proportional decrease in the hemoglobin content.

Erythrocyte counts below 4,000,000 or hemoglobin percentages below 70 are shown in Table III. In this series both the sedimentation index and fibrin

TABLE III

SEDIMENTATION INDEXES AND FIBRIN VALUES ASSOCIATED WITH EITHER LOW ERYTHROCYTE COUNTS OR LOW HEMOGLOBIN PERCENTAGES

CASE	DIAGNOSIS	AGE AND SEX	ERYTHROCYTES MILLIONS	HEMOGLOBIN, PER CENT	SEDIMENTATION INDEX	FIBRIN, MG PER CENT
1	Old Pott's fracture	35F	3.89	68	18	260.5
*2	Menopause	42F	3.39	60	10.1	474.5
**3	Purpura hemorrhagica menorrhagia	36F	3.06	57	8.5	393.3
4	Generalized arteriosclerosis	70M	3.96	70	10.3	377.7
5	Fibromyomas	50F	3.31	40	6.4	300.0
6	Secondary anemia (origin not found)	42F	3.17	45	9.7	362.1
7	Diffuse cystitis	57F	3.94	68	6.8	366.6
8	Menorrhagia, secondary anemia	27F	3.94	16	8.5	387.5
9	Generalized tuberculosis	35M	4.27	65	14.4	469.4

\*Blood pressure systolic 156 and diastolic 94

\*\*Blood pressure systolic 170 and diastolic 96

content of the blood were determined. All have increased sedimentation indexes and fibrin values. At first glance it would seem that there is a distinct correlation between increased sedimentation index, increased fibrin and decreased erythrocytes and hemoglobin. More careful examination of the hemoglobin and erythrocytes, with the diagram, will dispel any idea that they can be correlated. In this series there was only one condition in which an increase of fibrin would be expected, and that was in Case 9. In view of the fact that this patient had generalized tuberculosis with extensive pulmonary involvement, it is to be expected that the blood would show an increased fibrin content as well as a high sedimentation index regardless of the blood picture.

which in this case was normal with the exception of a slight decrease in the hemoglobin percentage

Cases 3 and 6 are both cases of primary disease of the blood in Case 6 secondary anemia must be considered potentially a blood dyscrasia since the cause of the anemia could not be found. The hemoglobin and erythrocyte count in both cases are below normal. The sedimentation indexes and the fibrin content of the blood are high. The increased sedimentation index in these

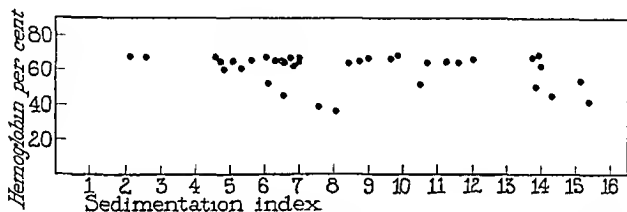


Fig 2—The relation between the hemoglobin per cent (Dare) of the blood and the sedimentation index

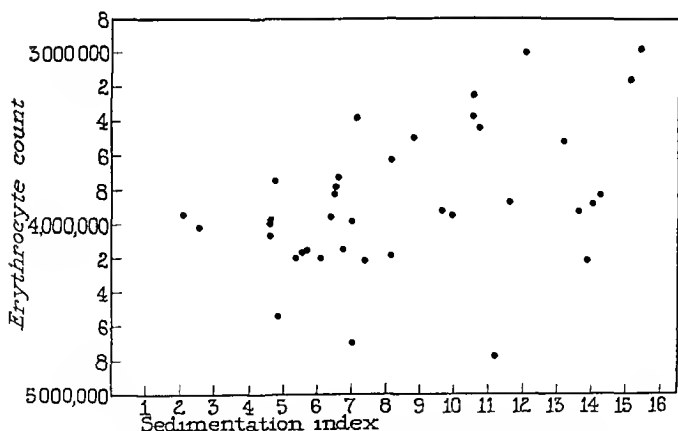


Fig 3—The relation between the erythrocyte count and the sedimentation index

cases can be explained by increased fibrinogen of the blood and by diminished blood count. In Case 4 the erythrocyte count is only slightly diminished, hemoglobin is normal and the fibrin content of 377.7 has a sedimentation index higher than either Case 3 or 6. Cases 1, 2, 5, 8 and 9 show similar variations if compared with each other or the ones already mentioned. The only correlation that can be made is that in all of the cases the cell count is decreased and the sedimentation index and fibrin are increased. The reason for this

may be that these cases were primarily some other condition associated with abnormal fibrinogen values with the development later of secondary anemia.

The sedimentation index was determined in 36 cases with maximal hemoglobin percentages of 70 and maximal erythrocyte counts of 4,000,000. Fibrin determinations were not made in this series. As in the previous series a definite ratio between the erythrocyte count and the sedimentation index could not be obtained. These data agree with those described by Groedel and Hubert, and Rubin and Smith.

Fig. 2 illustrates the relation between the hemoglobin and sedimentation index and Fig. 3 the erythrocyte count and the sedimentation index of the cases shown in Table II. To a certain extent, a decrease in the hemoglobin content of the blood parallels an increasing sedimentation index. Not infrequently, however, high hemoglobin values are found with high sedimentation indexes, and low hemoglobin values with low sedimentation indexes. The decrease in the hemoglobin is usually accompanied by a diminished number of erythrocytes, and this possibly more than the diminished hemoglobin accounts for the increased sedimentation index.

The variation of the cell count to any great extent either above or below normal limits affects the sedimentation index. By decreasing the cell volume (which would decrease the cell count) normal sedimentation becomes more rapid. Reversing this process normal sedimentation becomes slower.

The illustrations do not show exactly how much the cell count can vary without affecting the sedimentation index. Fig. 3 shows that as the blood count decreases, the sedimentation index increases but a definite parallelism is not shown between the two. Influences other than variations of the cell count affect the sedimentation index. For this reason the use of the index as a diagnostic measure in cases of anemia cannot be relied on.

#### ARTHRITIS

Gram<sup>22</sup> found hypermnesia in cases of chronic polyarthritis. He suggested that an explanation of this phenomenon may be sought in irritation of the fibrinogen-producing organs by the foreign proteins which have not passed the alimentary canal.

Fabricsius observed high sedimentation figures in three cases of arthritis deformans. He did not attempt to explain why this sedimentation occurred and was surprised that the high values were obtained in such chronic conditions.

Alexander in a series of 25 cases of chronic arthritis found sedimentation to be increased in 8. In none of the cases was the temperature elevated; all of the patients were ambulatory. In 6 of the 8 cases in which sedimentation was increased there was later a marked tendency toward acute exacerbation, and during exacerbation the sedimentation rate was accelerated. He concluded that increased sedimentation of the erythrocytes might indicate that the inflammatory process had not entirely subsided, and acute exacerbation might be expected.

Waggh and Nees both stated that the sedimentation reaction was normal in arthritis deformans.

The sedimentation indexes and the fibrin content of the blood were determined in three cases of arthritis. The sedimentation indexes as well as the

fibrin values were all above normal. In one case the patient had been menstruating three days. The blood counts and hemoglobin and the blood pressure in all three cases were normal, thus showing that the increase in the sedimentation indexes must be primarily due to hyperinosis.

The sedimentation index was determined in 21 cases diagnosed as arthritis. In about half of this series the sedimentation index was increased. As the blood counts and hemoglobin values were all practically normal, the elevation of the sedimentation index cannot be attributed to the anemia.

From the general examination one could hardly make a distinction between cases in which sedimentation values were normal and those in which they were higher. In the presence of normal sedimentation there was a tendency toward acute exacerbation, as in the presence of abnormal sedimentation indexes. Although there is a tendency in some cases of arthritis for an increase in sedimentation, I do not see how the test could be of any value in this condition either from a diagnostic or prognostic standpoint.

#### TUMORS

It has never been proved that a malignant growth per se will cause hyperinosis. A tumor obstructing the bile ducts and producing intense icterus may cause distinct hyperinosis, although infection and ulceration of the growth is the usual cause. This is substantiated by Foster and Whipple who have shown that injury to any tissue excepting the liver will cause an increase of fibrin in the blood.

If a tumor, either benign or malignant, becomes infected with disintegration and reabsorption of the cell products, two conditions may be produced that will result in acceleration of the sedimentation of erythrocytes: an increase in the fibrin content of the blood and a decrease in the number of erythrocytes. The reports in the literature as to the value of the sedimentation index reaction in the diagnosis of malignant growths are conflicting. Some workers found the sedimentation of the erythrocytes to be increased in cases of malignant growth and others found the reaction normal.

I determined the sedimentation indexes in 21 cases of benign and malignant tumors and did not observe any specificity of the sedimentation index.

#### GYNECOLOGIC LESIONS

That hyperinosis is produced by pregnancy and acute suppurative processes with a corresponding increase in the sedimentation index has been shown. The test has been used extensively and satisfactorily as an adjunct in gynecologic diagnosis.

Linzenmeier found it particularly valuable in the differential diagnosis of ectopic gestation and diseases of the adnexa. He stated that as long as the rate increased, virulent bacteria may be present and hence he advises delivery in operation.

Haselhorst concluded from the observation of several hundred tests that the sedimentation index reveals the process of inflammation before the temperature or the leucocytes show any change.

Joseph and Marcus, Schumacher and Vogel and Rothe pointed out that

accelerated sedimentation is usually present in association with increased reabsorption of waste products as the result of inflammatory processes.

Friedlaender noted a speeding up of the sedimentation reaction in all infectious processes. He considered the test of little value as an aid in the differential diagnosis of pelvic lesions although it can be used to determine whether a patient with inflammatory adnexal disease, but with a normal temperature and a normal blood count, should be subjected to operation.

Bronnikoff, Falta, Nitschmann and Silber substantiated Friedlaender's conclusions as regards the infectious process. Falta, Nitschmann and Silber consider the test of doubtful value in the differential diagnosis of extruterine pregnancy and tumor of the adnexa.

The use of the test in the differential diagnosis of simple noninfected fibromyomas and a three or four months' fetus when the usual signs of pregnancy were lacking, might be of some value.

The literature seems to show that the test is of practical value in determining the time of operation. During the process of destruction of tissue and cells, hyperinosis occurs, the approximate degree can be determined by the sedimentation index (provided other factors that cause an increased sedimentation index can be eliminated), and so by deductive reasoning the degree of activity of the infectious process can be determined.

In the presence of chronic pelvic infections and in the absence of constitutional signs such as elevation of temperature and leucocytosis, the test is of little value. The sedimentation index may be only slightly, if any, increased, and this may be due to some of the factors mentioned or to a subacute inflammatory process elsewhere.

As a diagnostic aid in pelvic lesions the sedimentation index is not a specific test, it seems to reflect the general condition of the patient. What has been said about the use of the test in gynecologic conditions holds true in general surgical conditions. The degree of sedimentation is not specific in any one group of cases and is to be regarded in the light of a general reaction such as fever and leucocytosis rather than a characteristic test.

#### DISEASES OF THE THYROID GLAND

Little can be gleaned from the literature on the use of the sedimentation index in diseases of the thyroid gland. Waugh, Alexander and Nees observed that in hypothyroidism and hyperthyroidism sedimentation values were normal.

In the 500 cases in this series in which the sedimentation determinations were made, 46 cases were diseases of the thyroid gland. The sedimentation index varied from low to very high. In the cases in which determinations of fibrin were made a diagnosis of exophthalmic goiter was made in three. In two of these the fibrin values were rather high with correspondingly high sedimentation indexes. The other case was mild exophthalmic goiter and the determination of fibrin and the sedimentation index were normal.

It is hard to explain the great variations of the sedimentation indexes in diseases of the thyroid gland. Kottmann reported finding a decrease in the fibrin content of the blood. The number of my cases in which determinations of fibrin were made is too small from which to draw conclusions. I did not find a correlation between the sedimentation index and the severity of the dis-

case. Other factors that influence sedimentation were noticed, for instance, in a few of the cases the cell count decreased. Two patients were pregnant, and two were menstruating. These complicating conditions alone might have caused elevation of the sedimentation index.

Elevation of the sedimentation index in certain diseases of the thyroid gland, especially exophthalmic goiter, is apparent, but without more relation between the sedimentation index and the severity of the symptoms the test could not be of either diagnostic or prognostic value.

#### HYPERTENSION

While classifying the results obtained from determinations of sedimentation I noted a large group of cases of hypertension in which sedimentation indexes were high. After a more careful scrutiny of the entire series I also noticed a number of cases of secondary hypertension in which the sedimentation index was high. In the cases in which determinations of fibrin were made I noted four of hypertension the sedimentation index in all was high.

In explaining the high sedimentation values in these cases the usual factors, such as hyperinosis or diminished blood count, must be considered.

Gram determined the fibrin in the blood of cases of nephritis with hypertension. In a large percentage of his cases the blood and plasma fibrin were definitely increased. He attributed hyperinosis to secondary infection, predisposed by stasis and edema of the lungs, or to the underlying infection or the toxic effect of organisms in the kidney.

It does not seem possible that in chronic nephritis with hypertension but without pulmonary involvement the infection in the kidney could produce such marked hyperinosis as was found in some of the cases in my series.

Lander, Lundsgaard and Van Slyke in studying the plasma proteins in nephritis, found that in the presence of nephrosclerosis (Volhard and Fahr classification of nephritis) the plasma proteins were not decreased. In the vascular interstitial type the effect was the same as in nephrosclerosis but in the glomerulo tubular or nephrotic type active or recently active there was a decrease in the plasma proteins. Fahr and Swanson confirmed the work of these investigators and also reported an increase of the plasma fibrin in practically all of the cases studied.

I will not attempt to discuss the relationship between nephritis and hypertension. Suffice it to say the relationship between these two conditions has puzzled investigators since the time of Bright. Many theories have been advanced but according to some authorities we are no nearer a solution of the problem than we were fifty years ago.

That hyperinosis is present in the blood of patients with nephritis has been shown. As most patients with hypertension have more or less renal involvement the resulting hyperinosis is expressed by variations of the sedimentation index. Whether or not the sedimentation test is of any value in determining the amount of renal involvement in cases of hypertension remains to be seen.

#### SUMMARY

A critical study of methods employed in the sedimentation test has been made. By combining and modifying several tests a simple and expedient test

has been devised. A simple method of reporting results has been proposed, the results being given in terms of a sedimentation index. Average normal values for men are from 0.1 to 2.0 and for women from 1.1 to 3.4, although in occasional instances there are unexplained variations from the average values. Sedimentation tests by this method can readily be compared with those obtained by other methods.

It has been demonstrated that the fibrin content of the plasma, as well as the sedimentation index, is higher in women than in men.

An increase in the plasma fibrin and the sedimentation index is usually present in infancy and early childhood, in old age during menstruation and during pregnancy.

In pathologic conditions the parallelism between the increase in the fibrin content of the blood plasma and the increase in the sedimentation index of the blood has been shown.

The sedimentation index is of little value in the diagnosis of pregnancy. In the early months the index may not be altered, while in the later months of pregnancy other signs are so marked that the test is not necessary.

The sedimentation index may be of value in determining the degree of activity of a tuberculous process provided cell volume does not diminish. As a primary diagnostic aid there is little to be gained from its use.

Reduction of the cell volume results in an increased sedimentation index, although there is no constant ratio between the results obtained. The use of the sedimentation index as a diagnostic measure in cases of anemia cannot be relied on.

In certain cases of arthritis the sedimentation index is elevated. Variations in either the fibrinogen content or the cell volume may be responsible for this increase. As a diagnostic or prognostic procedure, the test does not have any value.

Either benign or malignant tumor may produce a high sedimentation index depending on the site of the tumor, the presence of infection, the amount of destruction of tissue, or the degree of anemia.

It has not been proved that malignant growths per se will cause an elevation of the sedimentation index. For this reason the test cannot be of use in the differential diagnosis of benign and malignant growths.

As an aid in the differential diagnosis of gynecologic lesions, the sedimentation test has proved to be of some value. It has also been used to determine the time for operating on pelvic lesions of inflammatory origin.

High sedimentation indexes are found in some cases of exophthalmic goiter, and can usually be explained by the anemia present. The test is not of value in determining either the type or degree of hyperthyroidism.

In four cases of hypertension, hyperkalemia was noted. With a few exceptions cases of hypertension gave high sedimentation indexes. It is suggested that the degree of nephritis in these cases is responsible for the hyperkalemia and the resulting elevation of the sedimentation indexes. It is also suggested that the test may be of value in determining the amount of renal involvement in cases of hypertension.

## CONCLUSIONS

1 Variations of the fibrin content of the blood or of the cell volume (as expressed by the erythrocytic count) exert a marked influence on the sedimentation reaction. It must be admitted that factors other than those mentioned play a part in causing the sedimentation of erythrocytes.

2 During menstruation and pregnancy the fibrin content of the blood increases, and the sedimentation index is higher.

3 The test is known to be of practical value (a) in determining the degree of activity of a tuberculous process (b) as an aid in the differential diagnosis of gynecologic lesions and (c) as an index of the degree of activity present in pelvic infection. In all of the conditions mentioned there is no degree of specificity in the test and the clinical and laboratory data must be carefully considered.

4 In cases of hypertension sedimentation indexes are usually high. Whether in these cases the use of the test is of value in determining the amount of renal involvement remains to be seen.

## REFERENCES

- 1 Alexander M E. Clinical and Experimental Observations on Blood Sedimentation. *M J & Rec* 119 549 552 1924
- 2 Baer J L and Reis R A. The Sedimentation Test in Obstetrics and Gynecology. *Surg Gynec Obst* 40 691 696 1925
- 3 Bergeron Emil. Ueber Fibrinausscheidung beim gesunden und kranken Kinde nebst Analysen normalen und pathologischen Blutes. *Arch f Kinderh* 18 178 197 189
- 4 Bochner W and Wassing H. The Blood Sedimentation Test (Fährrens) in the Diagnosis and Prognosis of Disease. *J Lab & Clin Med* 11 214 216 1925
- 5 Brannikoff X. Zur Frage über die Methode der Blutkörperchensenkungsreaktion und ihrer Bedeutung in der Gynäkologie. *Zentralbl f Gynäk* 48 1483 1489 1924
- 6 Chandler, Joseph. The Determination of Fibrin in Blood Plasma. *J Lab & Clin Med* 12 1092 1096 1926 1927
- 7 Cooper, H N. Sedimentation Rate of Red Blood Cells. *J Lab & Clin Med* 11 615 623 1925 1926
- 8 Do Lee J B. Principles and Practice of Obstetrics. Philadelphia W B Saunders and Co., 1924 p 107
- 9 Dochez A R. Coagulation Time of the Blood in Lobar Pneumonia. *J Exper Med* 16 693 700, 1912
- 10 Dreyfus W and Hecht P. Ueber die Bedeutung der Senkungsprobe der Roten Blutkörperchen für die prognostische Beurteilung der chronischen Lungentuberkulose, München med Wchnschr 1 775 776 192
- 11 Drinker, C K and Hurwitz S H. The Factors of Coagulation in Primary Pernicious Anemia. *Arch Int Med* 15 733 745 1915
- 12 Erben, Franz. Die chemische Zusammensetzung des leukämischen Blutes. *Ztschr f klin Med* 66 278 301 1908
- 13 Fahr George and Swanson W W. The Quantities of Serum Albumen Globulin and Fibrinogen in the Blood Plasma in Acute and Chronic Nephropathies. *Arch Int Med* 38 510 526, 1926
- 14 Fährrens Robin. The Suspension Stability of the Blood. *Acta med Scandinav* 55 1298 1921
- 15 Falta Béla. Über die Verwendung der Senkungsgeschwindigkeit der roten Blutkörperchen in der Geburtshilfe und Gynäkologie. *Zentralbl f Gynäk* 48 1478 1483 1924
- 16 Foster, D P. A Clinical Study of Blood Fibrin With Observations in Normal Persons, Pregnant Women, and in Pneumonia and Liver Disease. *Arch Int Med* 34 301 312, 1924
- 17 Foster, D P and Whipple G H. Blood Fibrin Studies. IV. Fibrin Values Influenced by Cell Injury Inflammation Intoxication Liver Injury and the Eck Fistula. *Am J Physiol* 58 407-431 1921
- 18 Friedlaender Bernhard. Blood Sedimentation Test as an Aid in Diagnosis in Surgical Infections, *Am J Obst & Gynec* 7 12 144 1924
- 19 Frisch A. Ueber Blutweissuntersuchungen bei Tuberkulose. *Beitr z Klin d Tuberk* 48 145 169 1921



- 20 Grife, L Zur Differenzialdiagnose der Lungentuberkulose vermittle der Bestimmung der Sedimentierzeit der Erythrocyten, *Klin Wehnschr* 1 937 938, 1922
- 21 Gram, H C On the Causes of the Variations in the Sedimentation of the Corpuscles and the Formation of the Crusta phlogistica ("Sieve," "Buffy Coat") on the Blood A Preliminary Communication, *Arch Int Med* 28 312 330, 1921
- 22 Gram, H C The Results of a New Method for Determining the Fibrin Percentage in Blood and Plasma, *Acta med Scandinav* 56 107 160, 1922
- 23 Groedel, F M, and Hubert, G Die Blutkörperchensenkungsgeschwindigkeit bei inneren Erkrankungen, besonders bei Kreislaufstörungen, *Ztschr f klin Med* 102 31 45, 1925 1926
- 24 Halliburton, W D A Textbook of Chemical Physiology and Pathology, London, Longmans, Greene and Co 1891, p 306
- 25 Haselhorst, G Praktische Brauchbarkeit der Senkungsprobe der Erythrozyten in der Gynäkologie nebst Untersuchungen über Fehlerquellen des Verfahrens, *Deutsche med Wehnschr* 2 1100 1102, 1922
- 26 Hurst, J C, and Long, C F The early Diagnosis of Pregnancy by Methods of Precision Further Observations on Sugar Tolerance Tests Final Report, *Am J Med Sc* 171 846 853, 1926
- 27 Hunt, H F Studies of Sedimentation of Erythrocytes, *J Lab & Clin Med* 13 327 333, 1928
- 28 Hurwitz, S H, and Drinker, C K The Factors of Coagulation in the Experimental Aplastic Anemia of Benzol Poisoning With Special Reference to the Origin of Prothrombin, *J Exper Med* 21 401 421, 1915
- 29 Joseph, S, and Marcus, M Die klinische Bedeutung Senkungsgeschwindigkeit der roten Blutkörperchen als differentialdiagnostisches Hilfsmittel bei akuter Appendizitis und Adnexitis, *Med Klin* 1 607, 1923
- 30 Kollmann, Paul Zur Aetiologie und Therapie der Eklampsie, *Zentralbl f Gynäk* 21 341 346, 1897
- 31 Kottmann, K Beiträge zur Physiologie und Pathologie der Schilddrüse, *Ztschr f klin Med* 71 362 368, 1910
- 32 Krosing, L Das Fibrinogen im Blute von normalen Graviden, Wöchnerinnen und Eklampsischen, *Arch f Gynäk* 94 317 331, 1911
- 33 Lakschewitz Quoted by Fihrens
- 34 Landsberg, Eriek Untersuchungen über den Gehalt des Blutplasmas in Gesamteiweiß, Fibrinogen und Reststickstoff bei Schwangeren, *Arch f Gynäk* 92 693 720, 1910
- 35 Levinson, S A The Suspension Stability Rate of Erythrocytes in Pulmonary Tuberculosis, *Am Rev Tuberc* 7 264 277, 1923
- 36 Lewinski, Johann Beobachtungen über den Gehalt des Blutplasmas an Serumalbumin, Serumglobulin und Fibrinogen, *Arch f d ges Physiol* 100 611 633, 1903
- 37 Lander, G C, Lundsgaard, C, and Van Slyke, D D The Concentration of the Plasma Proteins in Nephritis, *J Exper Med* 39 887 920, 1924
- 38 Lanzenmeier, G Die Blutkörperchensenkungsgeschwindigkeit als differentialdiagnostisches Hilfsmittel bei Adnoverkrankungen, *Zentralbl f Gynäk* 46 535 542, 1922
- 39 Mills, C A Blood Clotting and the Control of Hemorrhage, *Med Rec* 101 149 151, 1922
- 40 Morriss, W H The Value of the Erythrocyte Sedimentation Determinations in Pulmonary Tuberculosis, *Am Rev Tuberc* 10 431 440, 1924 1925
- 41 Nees, O R The Red Cell Sedimentation Test A Review of the Literature With a Report of Its Application in over 150 Cases, *U S Nav Med Bull* 23 471-477, 1925
- 42 Nitschmann Unsere Erfahrungen mit der Erythrozytensenkungsgeschwindigkeit in der Gynäkologie, *Deutsche med Wehnschr* 1 393 394, 1925
- 43 Pfeiffer, T Ueber den Fibringehalt des menschlichen Blutes und die Beziehungen desselben zur sogenannten Crusta phlogistica, *Ztschr f klin Med* 33 215 257, 1897
- 44 Pfeiffer, T Ueber den Fibrinogengehalt leukämischen Blutes, *Zentralbl inn Med* 25 809 816, 1904
- 45 Rothe, E Ueber den Wert der Blutkörperchensenkung in der Chirurgie, *Zentralbl f Chir* 50 1328 1332, 1923
- 46 Rubin, E H, and Smith, N N Relation of Hemoglobin, Cell Count and Cell Volume to the Erythrocyte Sedimentation Reaction, *Arch Int Med* 39 303 314, 1927
- 47 Sakne, F, and Tsutsumi, T On the Causes of Rapid Sedimentation of Red Corpuscles of Blood of Pregnant Women, *Japan Med World* 1 No 7, 13, 1921
- 48 Sakamoto, T Beiträge zur Kenntnis von Organextraktgiften und über die entgiftende Fähigkeit des Blutes für dieselben *Ztschr f Immunitätsforsch u exper Therap Orig* 32 1 65, 1921
- 49 Salomon, M, and Valtis, J De l'étude des variations du temps de sedimentation globulaire dans la tuberculose pulmonaire, *Presse méd* 1 644 646, 1925

- 50 Schumacher, Paul and Vogel Walter Die Bedeutung der Blutkörperchensenkungs geschwindigkeit für die Diagnostik gynäkologischer Erkrankung, Arch f Gynak 119 127 135 1923
- 51 Silzer, O Unsere Erfahrungen über die Bräuhbarkeit der Blutkörperchensenkungs geschwindigkeit in der Gynäkologie Zentralbl f Gynak 50 353 361 1926
- 52 Schultz, E W Nicholes J K, and Schaefer J H Studies on Blood Fibrin, Am J Path 50 101 115 1925
- 53 Waugh T R The Blood Sedimentation Test Its History, Technique Nature and Clinical Application Can Med Assn J 13 604 608, 1922
- 54 Wells, H G Chemical Pathology Philadelphia W B Saunders Co, 1920, p 305
- 55 Wells, H G Chemical Pathology Philadelphia W B Saunders Co 1920, p 299
- 56 Westergren A Studies of the Suspension Stability of the Blood in Pulmonary Tuberculosis, Acta med Scandinav 54 247 283 1920
- 57 Westergren A. Technique of Red Cell Sedimentation Reaction Am Rev Tuberc 14 94 101, 1926

## A STUDY OF RETENTION NEPHRITIS\*

By J R NAKADA BS MD, St Louis Mo

THE importance of the determination of inorganic phosphates of blood plasma in nephritis has already been stressed by a number of investigators among whom may be mentioned Gienewald,<sup>1</sup> Marriott and Howland and Denis and Minot.<sup>2</sup> Schmitz, Rohdenburg and Myers stated that the determination of inorganic phosphates of blood plasma is of definite prognostic value. DeWesselow<sup>4</sup> remarks that nitrogen retention per se is harmless and is only useful because it to some degree indicates the degree of kidney damage. On the other hand he believes that phosphate retention involves certain deleterious results. He observed that phosphate retention is a better guide in regard to immediate prognosis than is urea retention. It draws a sharper line between the fatal and nonfatal cases. In this paper I have attempted to show what importance could be attached to phosphate determinations on a series of nephritic and genical hospital cases.

### PROCEDURE

The patients studied in the present investigation were largely on the Medical Service of St Mary's Infirmary. The phosphate determinations were made by the Briggs' modification of the Bell-Doisy method. Prepare a filtrate by adding to one part of oxalated blood plasma three parts of distilled water and one part of 27 per cent trichloroacetic acid in an Erlenmeyer flask. Shake mixture, allow it to stand a few minutes, and filter through ashless filter paper. Into a 10 cc graduated cylinder deliver 5 cc of this plasma filtrate with a 5 cc volumetric pipette. To this add 1 cc of a 5 per cent ammonium molybdate solution in 5N H<sub>2</sub>SO<sub>4</sub> and 1 cc of a solution of 5 per cent acid sulphite and 1 per cent hydroquinone. Simultaneously prepare in another graduated cylinder a standard made up of 5 cc of a phosphate standard (containing 0.03 mg phosphorus), 1 cc of the molybdate and 1 cc of the acid sulphite.

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hydroquinone solutions. Add distilled water to each of the graduates up to the 10 c c mark, invert (with finger over the opening of the graduates) two or three times and allow to stand from fifteen to thirty minutes. Then compare under a DuBoseq colorimeter and calculate the mg of phosphates in the plasma.<sup>6</sup>

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.03 \times 100 = \text{mg phosphate per 100 c c}$$

The nonprotein nitrogen was determined on a portion of etrated or oxalated whole blood, before centrifuging for phosphate determinations, by the direct nesslerization method of Folin and Wu.<sup>7</sup>

TABLE I  
CASES WITH HIGH PHOSPHORUS AND HIGH NPN

CASE	PHOSPHORUS	NPN	RATIO	RESULT	DIAGNOSIS OF CAUSE OF DEATH
A- 10	170	136.0	1 170	D	Uremia
- 71	144	274.6	1 177	D	Uremia
- 73	141	500.0	1 354	D	Cardi Proc., Metas in Lungs Chr Neph, Myoc (A)
- 51	120	300.0	1 250	D	Uremia
- 15	114	150.0	1 131	D	Uremia
- 5	114	200.0	1 177	D	Uremia
B-144	100	177.0	1 177	D	Chr Neph, Chr Myoc
A- 38	80	150.0	1 187	D	Chr Neph, Chr Myoc, Bronchopneumonia (A)
- 32	78	110.0	1 141	D	Chr Neph, Cirrhosis of Liver
- 79	71	153.0	1 210	D	Chr Neph, Chr Myoc
- 9	58	96.0	1 165	D	Chr Diffuse Neph, Chr Myoc, Arterio (A)
- 8	57	140.0	1 247	D	Bilat Congen Cystic Kidneys (Pneumoperit)
- 3	57	120.0	1 211	D	Nephrosis, Chr Neph
- 92	55	40.0	1 77	D	Chr Myoc, Aneur of Aorta, Arterio
- 20	50	92.0	1 184	D	Chr Neph, Chr Myoc
-133	50	48.4	1 97	D	Chr Myoc
- 76	47	53.6	1 114	D	Chr Neph, Chr Myoc
- 40	46	66.8	1 147	D	Chr Myoc
- 12	46	53.1	1 115	D	Chr Neph, Chr Myoc
B- 69	46	41.4	1 89	D	Suppurative Nephritis (A)
-142	43	46.8	1 109	D	Chr Myoc, Auric Fib
A- 28	41	49.5	1 121	D	Chr Myoc, Chr Neph, Infarcts of Lungs (A)
- 77	41	54.6	1 135	D	Thromb Pulmon Vess, Encephalomalacia Arter (A)
A- 55	63	85.7	1 136	Unimproved	Chr Neph, Dental Caries
- 63	50	63.2	1 126	"	Chr Neph, Chr Myoc, Arterio
B-112	48	45.1	1 94	"	Chr Myoc, Auric Fib, Asthma, Carr Liver
B- 47	51	50.0	1 98	Improved	Ac Bronchitis, Angina Pectoris
- 61	50	41.3	1 83	"	Chr Neph, Intestinal Monilia
A- 59	50	40.0	1 80	"	Chr Neph, Chr Myoc, Lucs
B- 5	48	40.0	1 83	"	Chr Neph
- 93	48	40.0	1 83	"	Early Abortion, Endocervicitis
-132	46	42.9	1 93	"	Gon Arterio, Arterio Gang, Endart Obli
-100	46	40.0	1 87	"	Diab Mell, Chr Myoc, Mitral Regurg
-123	45	40.0	1 99	"	Partial Obstruc Gastro Enteros Opening
- 30	44	44.4	1 100	"	Chr Myoc
-130	43	40.0	1 83	"	Chr Neph, Chr Myoc, Auric Fib
- 72	42	41.4	1 97	"	Ac Bronch
-134	43	40.0	1 95	"	Hypertens, Obesity, Enlar Heart
- 98	41	43.0	1 104	"	Ac Rheu Fever
- 81	41	40.0	1 97	"	Simple Colloid Goiter, Chr Bronch

A signifies autopsy performed

Pneumoperit signifies pneumoperitoneum performed

For comparison a number of cases were included in the study which had no evidence of nephritis or cardiovascular disease. The average findings on these cases are phosphate 381 mg and nonprotein nitrogen 30.06 m. A phosphate value above 4 m. and nonprotein nitrogen of 40 mg or above were considered high. The results of the study of the various cases are given in the accompanying tables.

## RESULTS

The cases have been grouped according to the laboratory findings into several classes. Table I is composed of cases with nonprotein nitrogen of 40 mg or above and phosphate values greater than 4 m. It is found at once

TABLE II  
CASES WITH NORMAL PHOSPHORUS AND HIGH NPN

CASE	PHOSPHORUS	NPN	RATIO	RESULT	DIAGNOSIS OF CAUSE OF DEATH
B- 1	4.0	40.0	1 10.0	D	Nephritis
A- 67	3.8	54.5	1 14.3	D	Pneumonococcus Chr Myoc (A)
- 23	3.7	135	1 35.6	D	Chr Myoc Lues
- 11	3.7	72	1 15.2	D	Ac Fibrino Purulent Meningitis (A)
- 43	3.7	40.0	1 10.7	D	Chr Myoc
B- 37	3.7	40.7	1 10.9	D	Bulbar Pals; Gen Arterio
A- 30	3.4	44.4	1 12.9	D	Cereb Hemorr Chr Myoc
B- 38	3.2	80.0	1 24.7	D	Chr Myoc Arterio Chr Neph (A)
A- 21	2.2	68.0	1 21.7	D	C S Mening Septicemia As Ulcer Gastro E
A- 36	3.2	70.4	1 22.3	D	Lobar Pneu Arterio Chr Neph
- 48	2.8	46.3	1 16.2	D	Chr Myoc
- 32	2.6	50.0	1 19.1	D	Ac Periton Perforation of Intes
- 6	2.5	40.0	1 16.0	D	Lobar Pneu Chr Myoc Chr Paren Neph
- 18	2.5	03.8	1 2.5	D	Hemiplegia Chr Myoc (A)
- 31	2.5	40.0	1 16.0	D	Ulcerative Colitis Secon Anemia (A)
- 75	2.3	52.6	1 23.2	D	Pneumonia—Type III Septicemia (A)
- 72	1.6	52.2	1 33.0	D	Malignancy of Kidneys
B- 83	4.0	50.0	1 12.5	Unimproved	Enlarg Heart Chr Neph
- 57	4.0	40.0	1 10.0		Enlarg Heart Hypertens
A- 33	3.7	42.8	1 12.1		Chr Myoc Auric Fib
- 70	3.2	42.9	1 13.5		Lung Abscess
- 60	3.0	61.6	1 20.5		Chr Myoc Enlar Heart Asthma
- 19	2.2	44.4	1 19.9		Chr Myoc Auric Fib
A- 22	4.0	77.0	1 14.2	Improved	Hemiplegia Lues
B- 71	4.0	40.0	1 10.0		Chr Myoc
- 51	4.0	40.0	1 10.0		Enlar Heart Hypertens
- 9	4.0	40.0	1 10.0		Nephrothiasis
- 48	3.9	41.4	1 10.7		Enlar Heart Chr Bronch
A- 64	3.8	41.8	1 11.0		Chr Myoc Arterio Chr Neph
- 4	3.6	42.2	1 11.6		Cirr Liver Ac Cholecyst
- 14	3.5	53.3	1 15.1		Chr Myoc Chr Neph
- 2	3.4	40.0	1 11.7		Chr Myoc
- 20	3.3	43.8	1 13.2		Lues (Tert) GG Prostatitis
- 78	3.3	47.7	1 14.7		Lues (Tert)
- 77	3.2	48.0	1 15.2		Chr Bronch Chr Neph
- 78	3.1	40.0	1 12.9		Ac Fibrin Pleurisy Ac Bronch
- 60	3.0	61.6	1 20.5		Chr Myoc Enlar Heart Mit Regurg Asth Chr Neph
A- 13	3.0	73.3	1 17.7	Improved	Chr Myoc Chr Neph
- 3	3.0	42.1	1 14.0		Hemoptysis (Cause Undetermined)
- 44	3.0	40.0	1 13.3		Fissure in Ano Fistula in Ano

A signifies autopsy performed

that this table includes the vast majority of cases in which nephritic pathology seemed to predominate. Moreover, it is found that of all cases with nonprotein nitrogen greater than 50 and a phosphate value greater than 5, there was not a single case that left the hospital improved. Of the fourteen cases that fall in this group, thirteen died and one left the hospital unimproved. Moreover, in all of these cases a clinical diagnosis of nephritis was made, and in all except one case nephritis was the chief pathology present. In one case in which a prostatic carcinoma was also present the kidneys were found to be very badly damaged at autopsy, and a diagnosis of very severe chronic nephritis was clear. Five cases, all of which had phosphate values greater than eleven, were recorded as having died of uremia. An examination of the ratio of P/NPN in these cases shows that the increase in nonprotein nitrogen is greater than that of the phosphorus. The finding of unfavorable significance is, therefore, a phosphorus value greater than 5 and nonprotein nitrogen greater than 50. Those nephritics, who improved clinically, had in no instance a phosphate retention greater than 5.1.

TABLE III  
AVERAGE VALUES

(1) AVERAGE OF CASES WITH NPN OF 40 MG OR ABOVE								
Average Initial—				Average Terminal—				
	No Cases	Phos	NPN	Ratio	No Cases	Phos	NPN	Ratio
Died	40	5.4	89.7	1.163	15	5.4	94.0	1.175
Unimproved	11	4.3	50.2	1.117	8	3.9	68.4	1.149
Improved	34	4.2	49.8	1.117	11	4.0	35.8	1.90

(2) AVERAGE OF CASES WITH NPN BELOW 40 MG								
Average Initial—				Average Terminal—				
	No Cases	Phos	NPN	Ratio	No Cases	Phos	NPN	Ratio
Died	22	3.4	33.8	1.104	1	2.5	40.0	1.160
Unimproved	20	4.1	32.3	1.79	6	4.1	37.5	1.90
Untreated	4	3.5	31.2	1.88				
Improved	54	3.5	32.3	1.56	4	1.0	31.3	1.75

(3) AVERAGE OF CASES WITH RISING NPN WHO DIED								
Average Initial—				Average Terminal—				
Cases	Phos	NPN	Ratio	Phos	NPN	Ratio	Cause of Death	
B- 23	7.0	68.0	1.96	3.7	133.5	1.356	Nephritis	
-144	6.7	46.2	1.69	10.0	177.0	1.177	Nephritis	
- 38	6.7	66.6	1.100	3.2	50.0	1.247	Uremia	
- 20	6.3	68.0	1.108	7.0	92.0	1.184	Nephritis	
A- 33	6.0	67.5	1.112	5.7	120.0	1.2105	Nephritis	
- 71	4.0	90.0	1.225	14.4	254.6	1.177	Uremia	
- 38	3.7	100.0	1.267	8.0	150.0	1.187	Nephritis	
Sums	40.4	506.3	97.7	50.0	1007.1	1.538		
Average	5.7	72.3	1.139	7.1	143.8	1.209		

(4) AVERAGE OF CASES WITH IMPROVED NPN WHO DIED								
Average Initial—				Average Terminal—				
Cases	Phos	NPN	Ratio	Phos	NPN	Ratio	Cause of Death	
A- 52	8.0	150.0	1.187	7.8	110.0	1.141	Cirrhosis	
B- 39	7.0	53.4	1.107	3.0	30.0	1.100	Cirrhosis	
A- 43	4.3	60.0	1.139	3.7	140.0	1.107	Cerebral	
- 30	2.5	57.2	1.229	3.4	44.4	1.129	Hemorrhage	
- 72	1.8	54.6	1.310	1.6	52.2	1.330	Leucosarc	
							Kidneys	
Sums	21.6	375.2	1.972	19.5	276.6	1.807		
Average	4.3	75.0	1.194	3.9	55.3	1.161		

Table II shows cases with a normal phosphate and a high nonprotein nitrogen. In nine fatal cases the nonprotein nitrogen retention was greater than 50 mg per 100 c.c. In one of those cases a sarcoma of the kidneys was present. In all others an accompanying infection or a serious heart lesion was an important factor in the fatal termination of the illness.

#### SUMMARY

1. A phosphate retention greater than 5 mg per 100 c.c. coupled with a nonprotein nitrogen greater than 50 mg per 100 c.c. indicates severe renal damage.

2. Nonprotein nitrogen values greater than 50, unaccompanied by phosphate retention, are indicative of renal damage, but the immediate prognosis is less grave.

3. Uremic symptoms are most commonly seen in cases with high phosphate retention.

#### REFERENCES

1. Greenwald I. The Estimation of Lipoid and Acid Soluble Phosphorus in Small Amounts of Serum. *J Biol Chem* 21: 29, 1915.
2. Marriott and Howland. Phosphate Retention as a Factor in the Production of Acidosis in Nephritis. *Arch Int Med* 18: 708, 1916.
3. Denis and Minot. A Study of Phosphate Retention From the Standpoint of Blood Analysis. *Arch Int Med* 26: 99, 1920.
4. DeWesselow. The Immediate Prognosis in Nephritis. *Lancet* 2: 163, 1923.
5. Schmitz, Rohdenburg and Myers. Inorganic Phosphate and Calcium of Blood in Nephritis. *Arch Int Med* 37: 233, 1926.
6. Briggs A. P. Some Applications of the Colorimetric Phosphate Method. *J Biol Chem* 59: 255, 1924.
7. Folin and Wu. A System of Blood Analysis. *J Biol Chem* 38: 91, 1919.

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# LABORATORY METHODS

## A POTASSIUM FERRICYANIDE METHOD FOR THE DETERMINATION OF REDUCING SUBSTANCES PRESENT IN BLOOD\*

By HOWAR L. BRYANT, B.S., M.A., NEW YORK CITY

### INTRODUCTION

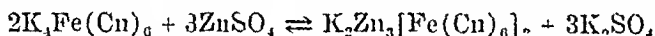
THE determination of the sugar in the blood of invertebrates and cold-blooded vertebrates by a copper method is frequently vitiated by a precipitation of the copper of the oxidizing agent by some substance in the filtrate. To obviate this difficulty the author sought to develop a method which would combine the use of the Folin and Wu<sup>1</sup> blood filtrate and the oxidizing agent of Hagedorn and Jensen.<sup>2</sup>

The principal disadvantages of the Hagedorn and Jensen method are the relative large error induced by the small amount of blood used and the incomplete precipitation of the blood proteins by zinc, while in many of the copper methods there is fairly rapid reoxidation of the reduced copper. It is thought that the method to be described avoids some of these difficulties.

### GENERAL PRINCIPLES

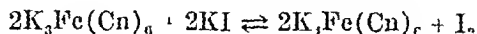
Potassium ferricyanide in a solution made alkaline with sodium carbonate is reduced to potassium ferrocyanide by certain of the reducing substances present in the blood filtrate.

In the presence of an excess of zinc sulphate the potassium ferrocyanide is precipitated. The reaction may be represented by the following equation:



The potassium zinc ferrocyanide is insoluble in acetic acid and the reaction is completed in one direction.

The unreduced potassium ferricyanide is later reduced by potassium iodide in a solution made acid with acetic acid. The reaction may be represented thus:



The amount of iodine set free is determined by titrating with standard sodium thiosulphate, and so becomes an indirect measure of the reduction brought about by substances in the blood filtrate.

### EXPERIMENTAL PART

*Precipitation of Proteins*—The blood proteins were precipitated by the technique described by Folin and Wu. An aliquot portion of 5 c.c. is used for the determination.

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*Solutions*—All the solutions used were made with Merck's reagent chemicals

*The Alkaline Potassium Ferricyanide Solution* was made by dissolving 3.29 gm of potassium ferricyanide and 85.5 gm of anhydrous sodium carbonate in distilled water and diluting to the final volume of one liter

G Kassner<sup>3</sup> reports that potassium ferricyanide solutions of alkaline earths are unstable. Sodium hydroxide causes it to deteriorate rapidly. The rate of this change of alkaline potassium ferricyanide solution is indicated in Table I. Light is an important factor in affecting the rate of change.

*A 5 Per Cent Zinc Sulphate Solution* was made with the salt which contained seven molecules of water of crystallization.

*A 10 Per Cent Potassium Iodide Solution*. This solution should be tested with starch and discarded if free iodine is found.

TABLE I

RATE OF CHANGE OF THE ALKALINE POTASSIUM FERRICYANIDE ON STANDING EXPOSED TO DAYLIGHT

NO OF DAYS	NO OF DETERM	BLANK DETERM N/100 c.c. THIO	MEAN DEVIATION	PERCENTAGE OF CHANGE
In Colorless Glass Bottle				
0	8	10.00	0.008	0.0
1	~	10.00	0.009	0.0
4	10	9.98	0.041	0.2
6	5	9.96	0.022	0.4
15	8	9.94	0.026	0.6
34	10	9.62	0.033	3.8
67	11	8.90	0.053	10.5
In Amber Colored Bottle				
0	4	10.02	0.030	0.0
98	5	10.02	0.023	0.0

*Determination*—General Discussion. Quantitative care should be exercised in making the protein free blood filtrate and the N/100 sodium thiosulphate, in pipetting the filtrate and the alkaline potassium ferricyanide solutions, and in the final titration. The making and pipetting of the potassium iodide, zinc sulphate and acetic acid solutions need not be so carefully done. Changes in temperature of the boiling water bath during the reduction reaction due to ordinary changes in atmospheric pressure produced no noticeable change in the final titration.

The zinc sulphate solution should be so delivered into the alkaline potassium ferricyanide that its rapid and uniform distribution is assured and precipitation of the potassium ferricyanide by the zinc is minimized. This may be accomplished by forcibly blowing the zinc sulphate solution from a pipette.

*Technic*. An aliquot portion of 5 c.c. of protein free blood filtrate is carefully pipetted into a test tube of about 20 mm x 170 mm. Ten c.c. of the alkaline potassium ferricyanide solution are added and the test tube is placed in boiling water for fifteen minutes. It is removed and then cooled at once by placing it in cold running water. After the solution is cooled the determination may be continued at any convenient time within three hours. See Table II.

Just before titrating 5 c.c. of zinc sulphate solution are added, then 2



cc of potassium ferricyanide solution and 5 cc of approximately 30 per cent acetic acid. The titration with N/100 sodium thiosulphate should be completed within the next minute or two. Two or 3 drops of 1 per cent starch solution are added immediately before the color due to the free iodine disappears. The end-point is the disappearance of the blue color.

The blank determination may be made in the following manner: a solution may be made which contains 1 volume of 10 per cent sodium tungstate, 1 volume of  $\frac{2}{3}$  N sulphuric acid, and 8 volumes of water. Five cc of this solution replaces 5 cc of the protein-free blood filtrate in the technique described above. Five cc of distilled water may also be used to replace the blood filtrate.

TABLE II

A COMPARISON OF THE EFFECT OF TIME OF STANDING ON THE AMOUNT OF REDUCTION OF POTASSIUM FERRICYANIDE SOLUTION BY GLUCOSE AT ROOM TEMPERATURE OF APPROXIMATELY 18° C

TIME IN HOURS	GLUCOSE ADDED MO	NO OF TITRATIONS	N/100 THIO CC	MEAN DEVIATION
0 0	1 50	12	4 54	0 032
1 0	1 50	6	4 55	0 041
2 0	1 50	6	4 35	0 027
3 0	1 50	16	4 58	0 036

TABLE III

A COMPARISON OF THE EFFECT OF THE MAXIMUM AMOUNT OF SODIUM TUNGSTATE, 5 CC OF FOLIN AND WU FILTRATE CONTAINING THE INDICATED AMOUNT OF STANDARD GLUCOSE, AND OF NO SODIUM TUNGSTATE, 5 CC OF WATER ALSO CONTAINING THE INDICATED AMOUNT OF STANDARD GLUCOSE, ON THE REDUCTION OF 100 CC OF N/200 ALKALINE POTASSIUM FERRICYANIDE

NUMBER OF TITRATIONS	CC N/100 THIO	ACTUAL GLUCOSE ADDED MG	GLUCOSE PER 100 CC MG	MEAN DEVIATION	MEAN DEVIATION OF MEAN
Filtrate					
16	1 58	0 50	50	$\pm 0 055$	$\pm 0 015$
14	2 37	0 75	75	$\pm 0 054$	$\pm 0 015$
14	3 15	1 00	100	$\pm 0 028$	$\pm 0 008$
15	4 58	1 50	150	$\pm 0 043$	$\pm 0 011$
15	6 11	2 00	200	$\pm 0 042$	$\pm 0 011$
38	7 51	2 50	250	$\pm 0 038$	$\pm 0 007$
21	8 96	3 00	300	$\pm 0 054$	$\pm 0 012$
Water					
43	0 32	0 10	10	$\pm 0 042$	$\pm 0 0064$
34	0 86	0 25	25	$\pm 0 047$	$\pm 0 0081$
17	1 55	0 50	50	$\pm 0 035$	$\pm 0 0085$
12	2 32	0 75	75	$\pm 0 030$	$\pm 0 0092$
16	3 02	1 00	100	$\pm 0 037$	$\pm 0 0092$
12	4 54	1 50	150	$\pm 0 032$	$\pm 0 0092$
12	5 99	2 00	200	$\pm 0 034$	$\pm 0 0099$
27	7 42	2 50	250	$\pm 0 034$	$\pm 0 0065$
19	8 79	3 00	300	$\pm 0 002$	$\pm 0 0004$

*Calculation*—The amount of N/100 sodium thiosulphate required by the free iodine in the unknown is subtracted from the amount required by the blank. The difference is proportional to the amount of potassium ferricyanide that has been reduced by the reducing substances in the blood filtrate. Since the aliquot represents but 0.5 cc of blood, the result is multiplied by 2.

and by 100 to obtain the reducing power per 100 cc of blood. In Tables III and IV this has been done.

**Glucose Standard.** Table III is based upon results obtained from a sample of glucose furnished by the United States Bureau of Standards. The specific rotation was stated to be as follows:

$$[\alpha]_{5461\text{\AA}}^{20.0} = 62.032 + 0.0422p + 0.0001897p$$

Where  $p$  is the percentage concentration. Glucose Sample 2, prepared by Duggan and Scott,\* agreed with the above in its reducing power.

A carefully weighed amount of glucose was dissolved in a suitable volume

TABLE IV

AMOUNTS OF GLUCOSE AND GLUCOSE CONCENTRATION OF BLOOD CORRESPONDING TO VARYING AMOUNTS OF N/100 SODIUM THIOSULPHATE

N/100 THIOSULPHATE CC	ACTUAL GLU COSE INDICATED MG	GLUCOSE IN 100 CC OF BLOOD MG	N/100 THIOSULPHATE CC	ACTUAL GLU COSE INDICATED MG	GLUCOSE IN 100 CC OF BLOOD MG
0.10	0.03	7	4.50	1.49	298
0.20	0.06	1	4.60	1.52	304
0.30	0.09	18	4.70	1.55	310
0.40	0.12	24	4.80	1.59	318
0.50	0.15	30	4.90	1.63	326
0.60	0.18	36	5.00	1.60	332
0.70	0.21	42	5.10	1.69	338
0.80	0.23	46	5.20	1.73	340
0.90	0.27	54	5.30	1.76	352
1.00	0.30	60	5.40	1.80	360
1.10	0.34	68	5.50	1.83	366
1.20	0.37	74	5.60	1.86	372
1.30	0.41	82	5.70	1.90	380
1.40	0.45	90	5.80	1.93	386
1.50	0.48	96	5.90	1.97	394
1.60	0.52	104	6.00	2.00	400
1.70	0.55	110	6.10	2.04	408
1.80	0.58	116	6.20	2.07	414
1.90	0.61	122	6.30	2.11	422
2.00	0.65	130	6.40	2.14	428
2.10	0.68	136	6.50	2.18	436
2.20	0.71	142	6.60	2.21	442
2.30	0.74	148	6.70	2.25	450
2.40	0.78	156	6.80	2.29	456
2.50	0.81	162	6.90	2.32	464
2.60	0.85	170	7.00	2.35	470
2.70	0.89	178	7.10	2.39	478
2.80	0.92	184	7.20	2.42	484
2.90	0.95	190	7.30	2.46	492
3.00	0.98	196	7.40	2.49	498
3.10	1.03	206	7.50	2.53	506
3.20	1.06	212	7.60	2.57	514
3.30	1.09	218	7.70	2.60	520
3.40	1.13	226	7.80	2.64	528
3.50	1.16	232	7.90	2.68	536
3.60	1.19	238	8.00	2.71	542
3.70	1.22	244	8.10	2.7	550
3.80	1.26	252	8.20	2.79	558
3.90	1.29	258	8.30	2.82	564
4.00	1.32	264	8.40	2.86	572
4.10	1.35	270	8.50	2.89	578
4.20	1.39	278	8.60	2.93	586
4.30	1.42	284	8.70	2.97	594
4.40	1.45	290	8.80	3.00	600

of water. Twenty c.c. of this solution were diluted to 100 c.c. Five c.c. of the latter, corresponding to 1 c.c. of the original solution, were used for each determination.

*Results of the Work With Blood*—A few experiments were carried out with the Shaffer-Hartmann method and my method simultaneously in order to compare the oxidizing power of the two methods on the protein-free blood filtrate of the blood of different animals. Table V shows that my method constantly gives higher values for the amount of reducing substances present in the filtrate. There is no apparent stoichiometrical relationship between the two methods.

TABLE V

A COMPARISON OF THE SHAFFER-HARTMANN AND THE AUTHOR'S METHOD ON THE AMOUNT OF REDUCING SUBSTANCES IN THE PROTEIN-FREE FILTRATE OF THE BLOOD OF DIFFERENT ANIMALS

BLOOD	MG. OF GLUCOSE PER 100 CC. OF BLOOD						DIFFERENCE
	SHAFFER-HARTMANN			AUTHOR			
	NO. OF DETERM.			NO. OF DETERM.			
Cat (etherized)	(7)	274	± 5.4	(6)	311	± 2.0	37
Ox (fresh)	(4)	79	± 1.0	(4)	121	± 1.5	43
Ox (fermented)	(5)	24	± 1.9	(5)	44	± 1.5	20
Rabbit (fresh)	(10)	143	± 2.7	(10)	166	± 5.0	23
Alligator (fresh)	(6)	69	± 3.5	(4)	112	± 1.5	43

From the above results it appears that other substances than glucose are oxidized by the potassium ferricyanide. While this is disappointing in some measure, the difficulty is probably one of degree only for it is generally conceded that none of the methods in general use, except possibly the latest methods of Benedict and of Folin,<sup>1</sup> are strictly specific for reducing sugars.

The method is useful in spite of its lack of specificity for measuring changes in the concentration of the reducing substances, especially so when these changes are presumably due solely to changes in the concentration of glucose as in insulin studies or work involving the feeding or the injection of glucose. Its use, in place of one of the copper methods, is to be preferred in studying the blood of certain invertebrates and of cold-blooded vertebrates.

## REFERENCES

- 1 Folin, O., and Wu, H. *J. Biol. Chem.* 38: 81-111, 1919.
- 2 Hagedorn, H. C., and Jensen, B. N. *Biochem. Ztschr.* 135: 46-79, 1923.
- 3 Kassner, G. *Arch. d. Pharmacol.* 234: 333.
- 4 Duggan, W. F., and Scott, E. L. *J. Biol. Chem.* 67: 287, 1926.
- 5 Benedict, S. R. *J. Biol. Chem.* 68: 759, 1926.
- 6 Folin, O. *J. Biol. Chem.* 67: 357, 1926.

# THE BENZIDINE REACTION\*

## SOME OBSERVATIONS RELATING TO ITS CLINICAL APPLICATION

BY ROBERT G. BRAMKAMP, A. B., BANNING, CALIF.

THESE experiments were undertaken to determine the quantity of blood introduced into the alimentary tract necessary to give a positive benzidine reaction in the stool. The relation of the test to the diet was also considered.

The benzidine test is simple of application and is quite generally used in clinical practice. Its sensitiveness for blood arising from different parts of the alimentary tract is therefore of practical importance.

The only work relating to the quantity of ingested blood required to give a positive stool reaction is that of Abrahams<sup>1</sup> who after living on a hemo-globin and chlorophyll free diet found that 1 cc gave a positive reaction. His experiments were apparently not continued.

Ogilvie states that any exclusion of meats from the diet (except for large quantities of rare meat) is unnecessary. Other than these experiments there has been little done relating directly to the effect of diet upon the test for occult blood, although many of those who have worked with the test recommend that a meat free diet be instituted several days before the stool is to be examined.

### EXPERIMENTAL

I *Technic*—Three general methods were used.

1 (For standardizing the reagents) Two cc of the solution to be tested are mixed with 1 cc of half saturated benzidine in alcohol. Lyle, Curtman, and Marshall<sup>2</sup> advise against the use of alcoholic solutions, but with fresh preparations quite satisfactory bluish tints obtained. To this mixture are added 0.5 cc of glacial acetic acid and 0.3 cc of hydrogen peroxide (3 per cent). A blue zone at the line of junction of the peroxide with the other solution indicates the presence of blood. This test is not applied to fecal material. For the latter, the following were used.

2 (Recommended by O & R Adler) After preliminary ether extraction the stool is treated with about one third its volume of acetic acid and thoroughly mixed. The mixture is then extracted with another portion of ether. The ethereal extract is mixed with 1 cc of benzidine solution, and 0.3 cc of hydrogen peroxide is added. A positive reaction is evidenced by the appearance of a blue color at the line between the last two liquids.

3 The slide test of Wagner. A smear of the stool is made on a slide and heated for a short time. Upon the smear are placed two drops of acetic acid, followed by eight drops of benzidine solution and two of hydrogen peroxide. In the presence of blood the smear and liquid become bright blue.

the intestine is important in decomposing the blood to products which do not react to the benzidine test. Dialysis of the digestion mixtures gave no indication that any of the reacting substances could pass through a semipermeable membrane.

*c Bacterial Decomposition*—Two cc of 50 per cent blood in water were inoculated with three loopfuls of a heavy fecal suspension. After incubating for forty-eight hours the solution was diluted to 1/1250 of the original blood volume, and it was found that 0.1 cc of the solution just gave a positive reaction, therefore there had been no reduction of the reacting blood by bacterial decomposition such as would occur in the intestine.

These tests (summarized in Chart 3) indicate that blood liberated in the stomach would be digested and absorbed to a considerable extent on passing through the intestine so that a considerable hemorrhage might occur before a positive test for blood would be obtained in the feces. On the other hand,

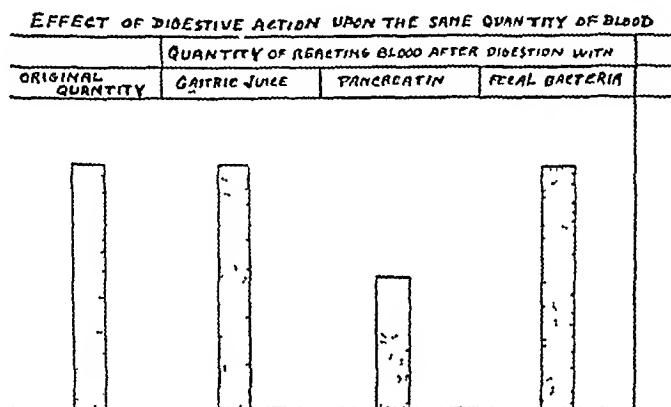


Chart 3

blood liberated in the lower part of the small intestine or in the colon is not appreciably acted upon, and therefore a much smaller amount would suffice to be detected by a stool test.

To test these assumptions we attempted to liberate the blood in the lower intestinal tract. One would expect to find that a much smaller amount would give a positive reaction in the feces. Dried blood was used, administered in stearic acid coated capsules. Two tenths gm of KI administered in these capsules appeared in the saliva only after three to four hours. This would allow the blood to be partially acted upon in the intestine but to a lesser extent than was permitted by the previous method.

The results of these tests are shown in Chart 2. Even though the stearic acid capsulation only partially reduced the intestinal digestion of the blood, there was a reduction of the quantity of blood required to give a positive stool reaction, to 1 gm (12 per cent weight of feces). It is to be assumed that a liberation of quite a small amount of blood in the lower small intestine or colon would result in a positive test in the feces. No suitable method was arrived at for the liberation of the blood so far down in the alimentary tract, so that the latter assumption could not be proved.

VI *Effect of Diet*—Many foods give a positive reaction to the benzidine test before ingestion. This is true of meats especially, and it is interesting to know to what extent the ingestion of these articles will affect the test for blood in the stools. The test negative diet employed in the previous experiments was also used here. To the daily diet the following were added with the results given below:

25 gm dried beef	positive
250 cc meat base vegetable soup	negative
100 gm chicken	"
1 lamb chop	"
70 gm hamburger	positive
40 gm liver (fried)	"
7 gm roast beef	"

It should be noted that these rather incomplete diet tests were performed on a single subject, and though he was apparently normal, there are individual differences in digestive power. The above diets would probably not be positive in most cases but they serve to emphasize that the diet of a patient should be inspected before a positive test for occult blood can be unquestioned. There seems to be no general agreement upon the relation of diet to the test and further experiments are necessary. One worker after an experiment conducted for eleven consecutive days' found no positive reaction, even after the ingestion of two ounces of boiled ox blood.

To be sure that a positive benzidine test in the stool is due to blood it seems advisable to at least require that all meat be omitted from the patient's diet for one or two days preceding the test. The following have been found to give stools that are consistently negative: milk, cheese, fresh eggs, well cooked potatoes, butter, dry breakfast foods, baked custard and most fruits. The patient when on the diet ingests a capsule containing 100 mg of carmine, which gives a distinctive color to the stool. A portion of the colored stool is then tested for occult blood. A positive reaction under these conditions is of definite diagnostic importance.

#### SUMMARY

1 Under the conditions of these experiments the ingestion of 35 gm of blood results in a positive stool reaction to the benzidine test. One gram of blood in a stearic acid capsule also gives a positive reaction.

2 In vitro pancreatic digestion reduces the reacting power of blood to one half the original.

3 Four hundredths per cent of fresh blood causes the stool to react positively. The liberation of this percentage of blood in the lower intestine should theoretically result in a positive test for occult blood, but this was not proved.

4 To obtain reliable results the patient should be placed on a meat free diet for two days preceding the test.

#### REFERENCES

- 1 Abrahams. Note on Test for Occult Blood. *Lancet* 2 490 August 21 1920
- 2 Ogilvie. Testing for Occult Blood in the Feces. *Brit Med J* 1 73 192
- 3 Lyle, Curtman and Marshall. A Study of Some of the Factors Involved in the Benzidine Test for Occult Blood. *J Biol Chem* 19 44 1914

# BENCE-JONES PROTEIN IN BLOOD SERUM LEADING TO DETECTION OF MULTIPLE MYELOMATOSIS\*

## REPORT OF A CASE

By J. J. SHORT, M.D., and I. R. CRAWFORD, M.D., New York

ON REMOVAL of sera from the inactivating water-bath for Wassermann test, one serum was noticed to contain a solid, gelatinous, white precipitate. This serum was notably clear before inactivation. Because of the temperature at which the precipitate had formed (56° C. for thirty minutes) the presence of Bence-Jones protein was suspected. Further examinations of the patient's urine revealed the presence of large quantities of Bence-Jones protein, these were followed by thorough x-ray examinations of his skeletal system which revealed in the cranium the usual pathologic changes found in myeloma. Details of the case follow.

*History*—W. W., male, aged fifty-two, born in the United States, real estate broker, married, applied for medical examination on March 26, 1927. His chief complaints were "sluggishness of intestines" and "weariness." Family history was irrelevant. Past history was that he had had chorea and typhoid fever, both with complete recovery. Habits were good except for the use of 6 cigars and 6 pipefuls of tobacco daily, little exercise, too free use of salt and pepper, with little fruit or vegetables in diet.

*Physical Examination*—The important findings were as follows. A large framed man, height 70 inches, weight 180½ pounds, appeared decidedly ill, worried, tired. Skin showed moderate pallor, eyes, ears, nose, throat and teeth were essentially negative, pulse 72, regular, blood vessels slightly thickened, blood pressure 98/60, heart normal in size, sounds lacking in tone, lungs negative, abdomen negative, reflexes negative, mental state apprehensive, extremities negative.

### LABORATORY FINDINGS

*Hemoglobin*—Light and eight tenths gm. per 100 cc. (equals 52 per cent, Williamson's standard), erythrocytes, 2,828,000, leucocytes, 6,000, color index, 0.91, polymuclear neutrophils, 57 per cent, lymphocytes, 35 per cent, endotheliocytes, 6 per cent, eosinophiles, 1 per cent, basophiles, 1 per cent. No parasites found.

*Urea Nitrogen*, 15.8, nonprotein nitrogen, 35.4, uric acid, 5.2, creatinine, 1.8, sodium chloride, 598, sugar, 105, cholesterol 150 mg. per 100 cc.

*Wassermann test* was negative with alcoholic and cholesterolized antigens.

*Routine Urinalysis*—Single specimen, amber, turbid, specific gravity 1.030, reaction acid, albumin trace, sugar negative, few epithelial cells, a little mucus and amorphous material. (In this examination the Robert's test was used for albumin and the Bence Jones protein was not detected.)

*Gastric Analysis*—Free HCl, 40, combined HCl, 16, organic acids and acid salts, 8, total acidity, 64, lactic acid, 0, occult blood, 0, microscopic, negative.

*Feces*—Essentially negative, except on one occasion a definite reaction for occult blood was obtained by the benzidine test which was not explained at the time. On a subsequent specimen the test was negative.

### ROENTGEN EXAMINATION

After obtaining the characteristic reaction for Bence Jones protein as noted above, numerous roentgenograms of the bones were made and reported as follows:

\*From the Laboratories and X-Ray Department of the Life Extension Institute, New York.  
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Examination of the entire skeletal system showed no changes suggesting multiple myeloma with the following exceptions. In the films of the cranium were found involving some of the bones of the cranial vault rounded areas of diminished density suggesting the possibility of multiple myelomatosis. In the bones of the right leg there was evidence of slight periostitis involving the tibia and to lesser extent the tibia of the left leg.

Unfortunately, several of the better original films were given into the possession of the patient and never recovered. One view of the cranial vault is reproduced in Fig. 1.



Fig. 1—Posteroanterior view of cranium showing isolated areas of diminished density at the apex of the cranial vault.

#### COMMENT

The patient continued at his work as real estate broker until about one month before his death in the spring of 1928 when he was confined to his bed. The immediate cause of death was extreme asthenia resulting from his progressive anemia. There were slight improvements in the blood count following several blood transfusions but the remissions were only temporary.

The two outstanding features of this case are the rather unique way in which the clue to the condition was first discovered and the degree of anemia incident to the slight degree of bone involvement. It would appear that the anemia was due to some inhibitory influence upon the marrow of the other bones resulting from the disease process as no extensive structural bone marrow change could be demonstrated.

#### REFERENCE

1. Williamson, C. S. Influence of Age and Sex on Hemoglobin. A Spectrophotometric Analysis of Nine Hundred and Nineteen Cases. Arch. Int. Med. 18: 50, 1916.



# THE USE OF SODIUM CITRATE AS ANTICOAGULANT IN THE CHEMICAL EXAMINATION OF BLOOD\*

By S L LEBORF M A, and I S WITCHELL M D, New York

**I**N THE chemical examination of blood, potassium oxalate is most widely used as an anticoagulant. It is the general impression that sodium citrate cannot be used, for the reason that it supposedly interferes with deproteinization, especially when tungstic acid is used as the protein precipitant. It is further claimed that the citrate interferes with the determination of uric acid.<sup>1</sup>

A study of the effect of potassium oxalate and of sodium citrate upon some of the chemical constituents of the blood was undertaken for the following reason: it is our intention to collect data on the distribution of calcium between corpuscles and plasma, and also the relation of calcium to other constituents of the blood. There is a question as to the presence of calcium in corpuscles, thus, Abderhalden<sup>2</sup> who examined the blood of the cow, bull, sheep, horse, pig, rabbit, dog and the cat, found no calcium in the corpuscles. He did not, however, examine the corpuscles of human blood for calcium. Marriott and Howland<sup>3</sup> also claim that there is no calcium in the corpuscles of human blood. They base their conclusion on their finding that whole blood contains approximately only half as much calcium as does serum. In opposition to this view are the following findings. Schmidt<sup>4</sup> claims to have found about 33 per cent of the calcium present in the corpuscles of human blood. Cowie and Calhoun reported the presence of calcium in the corpuscles of human blood in concentrations only somewhat smaller than in serum. Jones,<sup>5</sup> and Jones and Nye<sup>6</sup> give figures ranging from 50 to 87 mg. per 100 c.c. for the calcium content of the corpuscles of infants and young children.

It is obvious that oxalated blood cannot be used for the determination of calcium. Sodium citrate forms soluble calcium citrate and is the ideal anticoagulant with respect to calcium determination.

For the sake of economy it is desirable that the calcium determination be done on the same samples of blood that are being sent to the laboratory for routine chemical examination. Our routine examination consists in the determination of nonprotein nitrogen, urea-nitrogen, uric acid, and sugar. Other constituents are determined when requested.

## EXPERIMENTAL

Two hundred c.c. of blood were collected from a patient with pulmonary edema into two containers, 100 c.c. into each. One of these contained 30 drops of a 20 per cent solution of potassium oxalate, and the other contained 30 drops of a 25 per cent solution of sodium citrate. The concentration of the two salts was adjusted according to their molecular weights.

Complete prevention of coagulation was effected with both salts.

\*From the Biochemical Laboratory of Lebanon Hospital, New York.  
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The bloods were immediately taken to the laboratory and six 10 cc portions of each blood were pipetted into flasks. Varying amounts of oxalate and citrate were added to the respective bloods. To each sample was then added 70 cc of water and 10 cc of each, 10 per cent sodium tungstate and two thirds normal  $H_2SO_4$ . The following determinations were done on the filtrates: nonprotein nitrogen by the method of Koch McVeekin,<sup>8</sup> urea nitrogen by the method of Folin and Denis,<sup>9</sup> uric acid by the method of Folin,<sup>10</sup> creatinine by the method of Folin,<sup>11</sup> sugar by the method of Folin and Wu.<sup>1</sup> The results are shown in Table I.

TABLE I

NO BLOOD	ANTICOAG ADDED	NPN		UREA N		URIC ACID		CREATININE		SUGAR	
		OXL	CTR	OXL	CTR	OXL	CTR	OXL	CTR	OXL	CTR
1	None	36	36.0	16.2	16.9	18	3.9	17	16	113	111
2	2 dr	39	36.2	16.6	16.1	36	3.6	17	17	110	109
3	" "	36.6	36.1	16.1	15.8	37	4.1	16	15	112	109
4	10	36.4	37	16.9	16.4	*	3.8	18	16	109	106
5	1	36	41.0	16	17.0	37	3.7	17	18	10	102
6	1 cc	37.1	42.4	16.9	17.0	*	4.0	16	15	104	100

The solution became very cloudy and could not be read in the colorimeter.

## DISCUSSION

We encountered no difficulty in obtaining clear filtrates in the presence of citrate with the reagents used. In this respect the citrate and the oxalate behaved alike.

The citrate not only does not interfere with the determination of uric acid by the method used but as is shown by the table it is superior to the oxalate. The last three tubes containing large amounts of oxalate became very cloudy on adding the reagents and very little color developed while all the tubes containing citrate even those containing the largest amount used in the test remained very clear and developed a good color.

As for the other constituents determined in the presence of excess citrate the nonprotein nitrogen fraction is definitely increased while the amount of sugar is somewhat decreased. Practically this is of no significance since the amount of citrate producing such faulty results is far in excess of the amount that may ever be used for collecting blood.

The effect of sodium citrate on the chemical constituents of the blood was further tested on a number of bloods taken for routine examination. These

TABLE II

NO BLOOD	NPN		UREA N		URIC ACID		CREATININE		SUGAR	
	OXL	CTR	OXL	CTR	OXL	CTR	OXL	CTR	OXL	CTR
1	28.3	28.8	11.7	11.6	1	1.6	12	13	90	91
2	31.1	31.6	11.1	11.0	-	4	16	16	101	10
3	30.0	27	14.4	14.1	-	-	14	14	116	114
4	31	24.2	10.3	9.8	-	-	1	10	117	113
5	30.4	29.9	14.0	14.3	1.1	1.5	-	-	83	80
6	35.2	31	16.9	16.6	-	2.0	11	14	-	21
7	22.2	25.0	11.3	11.1	11	1.0	10	10	9	81
8	28.0	29.3	13	12.8	8	0.0	36	3	124	111
9	33.4	33.0	11.0	11.4	3.0	3.1	-	-	188	18
10	37.4	38.0	1	17.1	36	3	18	1	1	37

bloods were collected in duplicate, one portion in potassium oxalate, and the other portion in sodium citrate. The bottles for collecting the bloods were prepared by introducing three drops of 20 per cent oxalate solution into each bottle, and dried at a temperature of about 100° C. Another set of bottles was prepared containing three drops of 25 per cent solution of sodium citrate. These bottles were dried in the incubator at 37° C. overnight. High temperatures should not be used for drying the citrate.

The above-mentioned five determinations were then performed and the results compared. Table II shows that the results are quite satisfactory.

#### SUMMARY

1 A comparative study was made of the effect of potassium oxalate and sodium citrate upon the determination of some of the chemical constituents of the blood.

2 Sodium citrate may be used as the anticoagulant in blood for these determinations. With the amounts of citrate used no difficulties are encountered.

3 The complete precipitation of blood proteins is produced.

4 In the determination of uric acid by the method of Folin better results are obtained with the citrate than with the oxalate.

#### REFERENCES

- 1 Hawk, P. B., and Bergheim, O. *Practical Physiological Chemistry*, 1926. Blakiston's Philadelphia, p. 367.
- 2 Abderhalden, E. *Physiological Chemistry*, Translated by W. T. Hall and G. Derfen, 1911, Wiley and Sons, pp. 554-5.
- 3 Marriott and Howland. *J. Biol. Chem.* 32: 233, 1917.
- 4 Schmidt, Carl. *Charakteristik der epidemischen Cholera*, 1875, Leipzig und Mitau, pp. 29, 32. Quoted from A. P. Mathews, *Physiological Chemistry*, 1927, William Wood Co.
- 5 Cowie and Calloun. *J. Biol. Chem.* 37: 505, 1919.
- 6 Jones. *J. Biol. Chem.* 49: 187, 1921.
- 7 Jones and Nye. *J. Biol. Chem.* 47: 529, 1921.
- 8 Koch and McMeekin. *J. Am. Chem. Soc.* 56: 2066, 1924.
- 9 Folin and Denis. *J. Biol. Chem.* 11: 527, 1912.
- 10 Folin. *J. Biol. Chem.* 54: 153, 1922.
- 11 Folin. *A. Laboratory Manual of Biological Chemistry*, 1922, New York, Appleton and Co., p. 229.
- 12 Folin and Wu. *J. Biol. Chem.* 41: 367, 1920.

# THE WASSERMANN REACTION OF HUMAN MILK

## A COMPARISON OF BLOOD AND MILK TESTS IN 107 CASES

BY JULIUS JARCHO, M D F A C S,\* NEW YORK

THE performance of the Wassermann test on the milk suggests itself as a convenient method of detecting the presence of syphilis in puerperal women. To study the reliability of this test, specimens obtained from 125 mothers from the third to the seventh day postpartum were subjected to the Wassermann test in an institution in which the blood is examined routinely. In 107 instances a comparison of the Wassermann reaction in the blood serum and in breast milk showed close parallelism. The remaining 18 are not considered here since the reports of their blood are not available, of their milks 1 was three plus and 17 were negative.

### REPORTS OF EARLIER INVESTIGATORS

Bab<sup>1</sup> in 1907 performed Wassermann tests on the breast milk of three syphilitic and one nonsyphilitic woman and observed that the reaction was positive in the syphilitic patients and negative in the other woman. Later Gucciardi,<sup>2</sup> Bauer,<sup>3</sup> and Bar and Daunay<sup>4</sup> reported a few cases in which a positive reaction was obtained from the breast milk of women known to be syphilitic.

Bar and Daunay performed the Wassermann test on the breast milk of 8 women with florid syphilis. In 5 cases the reaction was positive in two, doubtful, and in one negative. In 15 nonsyphilitic women the reaction was negative.

Thomsen<sup>5</sup> in 1909 reported that in 9 syphilitic women who began to nurse their infants, the reaction of the breast milk was definitely positive during the first two days after labor. Then it began to diminish in intensity and usually became negative after the fifth or sixth day of the puerperium. In 8 syphilitic women who did not nurse their infants the positive reaction persisted until it was no longer possible to obtain milk for examination (8 to 14 days). In some of these women, known to be syphilitic, the Wassermann reaction of the serum was negative. In 82 nonsyphilitic women who were nursing their infants, the reaction was negative when the milk was tested from five to thirty-five days after labor. However, in 9 of 46 nonsyphilitic women whose milk was tested in the first two or three days of the puerperium the reaction was slightly positive until the fifth or sixth day. In none of these cases was the reaction of the serum positive. In 8 nonsyphilitic women who did not nurse their children, the reaction of the breast milk was uniformly negative.

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In a group of 28 puerperal or pregnant women, Rojas<sup>6</sup> in 1919 observed a positive Wassermann reaction in the milk or colostrum and also in the blood in 7 cases. Seventeen of the patients had been delivered within five days and 11 were in the last two months of pregnancy. In three of the positive cases, the reaction was more pronounced in the milk than in the blood. In 6 cases, there was a positive reaction in the milk and a negative one in the serum. The false positive reactions obtained in the colostrum may, in my opinion, be attributed to the richness of that substance in globulin or to the anticomplementary substances, if the milk had not been heated.

Examining the breast milk of 40 women, 16 of whom were known to have syphilis and 15 to be free from the disease, Rusea<sup>7</sup> in 1919 observed a constantly negative reaction in the mammary secretion from normal women from the second to the ninth month of pregnancy. In the secretions obtained from the syphilitic women there was a strongly positive reaction, which tended to disappear under treatment and parallel the reaction in the blood. Rusea's further study,<sup>8</sup> reported in 1921, confirmed the presence of syphilitic reagin in breast milk even in advanced lactation. He expressed the view that the milk reaction is not so sensitive as that of the blood, but that a positive reaction in breast milk indicates grave and active syphilis.

Klauder and Kolmer<sup>9</sup> in 1921 performed Wassermann tests on the breast milk of 19 syphilitic women, all of whom had a positive reaction of the blood. In the majority, the reaction was four-plus with three antigens. The majority of the patients were untreated and presented no clinical evidence of syphilis, although some had secondary and late secondary skin lesions.

The milk was obtained at variable periods after childbirth or during the last month of pregnancy. With the technique employed, fresh, unheated human milk was anticomplementary in amounts varying from 0.5 to 0.9 cc. Tests conducted with one-half or even slightly more of these anticomplementary amounts yielded negative reactions in the milk of all women except three, two of whom were tested within five days of delivery. In one of these two cases the milk was likewise negative before and three weeks after delivery. It is difficult to interpret the findings of Klauder and Kolmer because of the fact that raw milk was used and anticomplementary reactions were obtained, also because in 16 of the 19 cases the milk was examined a month or later after delivery.

In human milk from 89 syphilitic and 21 nonsyphilitic mothers, Cotellessa<sup>10</sup> in 1923 obtained parallel Wassermann reaction with those of the serum. To avoid inactivation the fat was removed by centrifugation. The test was performed with 0.5 and 1 cc. of milk.

Schwarz and Schubert<sup>11</sup> in 1925 reported parallel Wassermann reactions in the blood and milk of 45 women in the early days of the puerperium. In 17 cases the reaction in the blood was negative and that in the milk was also negative, except for an anticomplementary reaction in two cases. In the remaining patients, who were definitely syphilitic, the Wassermann reaction in the milk corresponded with that in the blood. However, there were some primary infections in which the reaction was positive in the blood but still nega-

tive in the milk. In three cases in which the blood reaction had been reduced by intensive treatment, the milk still gave a strongly positive reaction.

Martin<sup>1</sup> in 1925 noted that he had found the Wassermann reaction positive in the milk in all women with positive blood reactions, but in women nursing their infants he observed that the reaction becomes negative in a few days, thus confirming Thomsen's earlier report.

Hackemann<sup>12</sup> in 1926 reported a parallel study of the Wassermann reaction in the milk and serum in a number of cases. In 24 nursing mothers whose blood reaction was positive the reaction in the milk was also positive until the third to the eleventh day of the puerperium, an average of six to seven days. In 5 nursing mothers whose blood reaction was negative there was a positive reaction in the milk, which lasted until the third to the fifth day of the puerperium (average 4.5 days). In four cases the Wassermann was negative in both the blood and the milk. In three cases in women who did not nurse their infants the reaction was positive in the blood and also in the milk until the thirteenth to the twenty-fifth day postpartum. Hackemann believes that the false positives in the milk during the early days of the puerperium are due to the presence of serum globulin.

Lupinski and Keller<sup>14</sup> in 1926 reported a study of the Wassermann test on mother's milk in 200 women from which they concluded that the Wassermann reaction is positive in the milk of syphilitic women and remains so as late as the sixteenth day of the puerperium even in women who nurse their infants. In cases of latent syphilis they believe it may be positive even when the blood reaction is negative. In their experience the blood was never positive when the milk gave a negative reaction. In some cases they observed a false positive reaction during the first two or three days of the puerperium in nonsyphilitic women with negative blood tests but these false positives could be avoided by employing not more than 0.05 c.c. of milk for the test. In the case of syphilitic women the reaction was positive with 0.025 and even 0.0125 c.c.

Franken and Rothmann<sup>1</sup> in 1927 reported a study of 225 cases in which the Wassermann reaction of the milk was compared with that of the retroplacental blood and in some cases with that of blood obtained in the ordinary way. The Wassermann reaction in the milk agreed with that in the blood in 95.5 per cent of the entire series. In 11 cases in which there were no definite clinical signs of syphilis but the reaction of the arm blood and retroplacental blood was positive the reaction in the milk was also positive. In 4 cases in which the retroplacental blood was negative but there was either a definite history of syphilis or a positive reaction in the blood obtained from the arm the reaction in the milk was positive. In three patients under treatment for syphilis the milk gave a negative reaction. One of them had a positive blood Wassermann. In 8 cases in which there was no clinical evidence of syphilis and the blood test was negative, a nonspecific positive reaction was obtained in the milk. In 5 other cases without clinical evidences of syphilis, there was a nonspecific or anticomplementary reaction in both the milk and the serum. Franken and Rothmann conclude that the Wassermann test on the milk is of no more diagnostic value than that on the blood but that

it is of value as a check on the diagnosis in cases in which the retroplacental blood is not obtained or in which the blood from the amnion is not tested at the time of discharge from the hospital. Especially is this true in the detection of latent syphilis. In their experience, the Wassermann reaction in the milk of syphilitic women is most strongly positive from the eighth to the tenth day postpartum.

#### CLINICAL MATERIAL STUDIED

The milk was obtained from 125 recent colored mothers in a city institution in which the blood Wassermann is performed as a matter of routine. This class of patients was chosen because, by reason of social, economic and educational disadvantages, they would be less likely to resort to early medical aid when infected. Also, even if treatment were started early, this class of patients would be less likely to persist in the treatment sufficiently long to rid themselves of the disease than those in more fortunate circumstances.

Through the efforts of Dr. E. G. Langrock and the courtesy of Dr. F. A. Kassebohm, I was able to obtain milk from the third to the seventh day postpartum. Twice a week the milk was pumped or expressed from the breast. Before obtaining the specimens, the breasts were washed. A clean, sterile pump was used in each case. The specimens were placed in clean, sterilized saltcellar bottles and kept on the ice until examined. Each bottle was labeled with the following data: name, address, age, hospital number, number of children, number of abortions, day postpartum, result of blood Wassermann, result of milk Wassermann, and date when milk was obtained. Due in part to the fact that most of the patients were young primiparae ranging from sixteen to twenty-three years of age, the majority denied abortions. The youngest patient was thirteen years old, the oldest, forty-one.

#### REMARKS ON TECHNIC

The Wassermann tests on the milk were performed by Dr. A. A. Eisenberg, Director of the Sydenham Hospital Laboratories, who had no knowledge of the blood Wassermann results when he submitted his reports.

Milk may be prepared for the test by shaking with ether and prolonged centrifugalization or by passing it through a Berkefeld filter. The latter method, however, weakens the positive reactions somewhat, probably by holding back part of the reagent.

The technique of Franken and Rothmann,<sup>13</sup> which is almost identical with that of Lipinski and Keller,<sup>14</sup> is to be preferred. The milk is centrifugalized for thirty minutes at high speed, as a result of which three distinct layers were formed. The lowest layer is a thin stratum of grayish sediment, the middle layer, which is the largest, is watery and almost colorless, the upper layer is a narrow stratum of fatty material. A capillary pipette to which a rubber nipple is attached is introduced into the middle layer and the watery fluid drawn off. This fluid is inactivated by heating for thirty minutes in a water-bath and then diluted with normal saline solution in a ratio of 1:5 or 1:10. The amount of diluted fluid used for the test is from 0.05 to 0.1 c.c.

Thomsen's<sup>5</sup> observation that some nonsyphilitic mothers had Wassermann-positive milk is probably explained by the fact that he used noninactivated

milk, as was also the case in 8 of 185 cases reported by Lipinski and Keller.<sup>14</sup> Unheated milk may give a false positive due to the anticomplementary action of raw milk.

It is generally agreed that the milk should be obtained from nursing mothers from the sixth to the ninth day *postpartum* and that the reaction tends to become negative after the sixteenth day. The explanation probably lies in the fact that in the earlier days of the puerperium the breast secretion is more of the nature of colostrum than true milk, thus accounting for the close relation between its proteins and those of the blood serum. In true milk, on the other hand, the protein constituents are the product of a specific secretion of the mammary glands. Apparently the reagent responsible for the positive Wassermann reaction passes from the blood into the colostrum. This hypothesis would be consistent with the general observation that incomplete or slow emptying of the breast prolongs the duration of the positive Wassermann reaction in the milk. Schwarz and Schubert<sup>15</sup> sometimes observed positive Wassermann reactions in the milk of women who did not nurse their infants as late as the thirty second day after delivery because as they believed local congestion had caused a retention of colostrum.

#### RESULTS OF INVESTIGATION

The results of the parallel Wassermann tests on blood and milk respectively, in 107 cases are given in Table I.

TABLE I  
COMPARISON OF PARALLEL WASSERMANN TESTS ON BLOOD AND MILK

	NO OF CASES
Both blood and milk negative	86
Both blood and milk 4 plus	3
Both blood and milk 3 plus	3
Both blood and milk 1 plus	1
Blood 4 plus milk 3 plus	1
Blood 4 plus milk 2 plus	1
Blood 4 plus milk plus minus	1
Blood doubtful milk 1 plus	1
Blood 4 plus milk negative	1
Blood 1 plus milk negative	1
Blood negative milk 3 plus	1
Blood negative milk 2 plus	2
Blood doubtful milk negative	3
Blood negative milk anticomplementary	2
Total	107

If we drop from consideration the two cases in which the milk was anticomplementary, there was exact qualitative and quantitative agreement in 89 per cent of the cases and substantial agreement in 95 per cent.

In five cases contrary results were obtained. In three instances the milk was positive and the blood negative; in two the blood positive and the milk negative. The two anticomplementary reactions were due to the fact that unheated milk was used in these cases.



## PRACTICAL APPLICATIONS

If the Wassermann reaction of the breast milk of puerperal women closely parallels that of the blood, as the evidence strongly suggests, routine examination of the milk from the sixth to the ninth day postpartum should offer a convenient alternative to the blood Wassermann test. It is simpler and avoids unpleasantness in the case of sensitive patients.

A Wassermann test on the milk of prospective wet nurses, recently delivered, should be performed as a routine. The test is especially useful for women who refuse to submit to repeated blood tests though they may have become infected with syphilis and develop a positive reaction during their wet nursing period. All milk received at the stations should be subjected to the Wassermann test.

In private practice it is a simple matter for the physician to obtain a specimen of milk in suspicious cases and perform the test without the knowledge of the other members of the family. Many patients object to the blood test because it draws searching questions. The significant puncture mark of the Wassermann test has frequently attracted the attention of husbands or others and has thereby precipitated domestic crises. When the reaction in the milk is positive, a blood Wassermann should always be obtained for confirmation.

## SUMMARY

Parallel blood and milk Wassermann tests were performed upon 107 colored puerperal patients in a city institution. There was substantial agreement in 95 per cent of the cases, and exact qualitative and quantitative agreement in 89 per cent. In three instances the milk was positive and the blood negative, in two, the blood positive and the milk negative.

The Wassermann reaction remains positive in the milk of syphilitic women until about the sixteenth day, when it begins to weaken and gradually disappears. False positive reactions can largely be avoided by inactivating the milk and using smaller quantities for the test. Apparently the reaction is due to the presence of the reagin in the colostrum, whose composition more nearly approximates that of the blood serum than does the true milk.

Routine examination of the milk from the sixth to the ninth day postpartum offers a convenient means of employing the test in maternity practice.

## REFERENCES

- 1 Bab, H. Bakteriologie und Biologie der kongenitalen Syphilis, *Ztschr f Geburtsh u Gynak* 60 161, 1907
- 2 Guicciardi, G. Cited by Bai, P, and Daunay, R, *Recherches sur le sero diagnostic de la syphilis chez la femme enceinte et l'enfant nouveau né (méthode de Wassermann)*, *Obstetrique* 2 192, 260, 1909
- 3 Bauer. Cited by Thomsen, O
- 4 Bar, P and Daunay, R. *Ibid*
- 5 Thomsen, O. Wassermann'sche Reaktion mit Milch, *Berl klin Wchnschr* 46 2052, 1909
- 6 Rojas, D. A. La reaccion de Wassermann en la leche de mujer, *Semana med* 26 100, 1919
- 7 Ruser, C. L. Ricerche intorno alla sierodiagnosi di Wassermann sul latte muliebre, *Pediatrics* 27 343, 1919
- 8 *Ibid* Ulteriori osservazioni intorno alla sierodiagnosi di Wassermann sul latte muliebre, *Pediatrics* 29 121, 1921

- 9 Klauder I V and Kolmer J A Wassermann Test with Secretions, Transudates and Exudates in Syphilis, J A M A 76 1635, 1921
- 10 Cotellessa M Contributo allo studio della reazione di Wassermann sul latte di donna *Pediatrica* 31 860 1923
- 11 Schwarz G and Schubert M Ueber die Infektiosität der luetischen Frauenmilch *Arch f Dermat u Syph* 149 409 1925
- 12 Martin F Du Milch luetischer Wochenrinnen *Munchen med Wchnschr* 72 1205, 1925
- 13 Hackemann I Die Wassermannsche und die Floccungsreaktionen in der luetischen Milch *Munchen med Wchnschr* 6 773 3743 1926
- 14 Lipinski W, and Keller T Ueber den diagnostischen Wert der Wassermann'schen Reaktion in der Milch *Wicu med Wchnschr* 76 541 1926
- 15 Franken H and Rottmann, H G Ist die Vornahme der Wa R in der Milch der Wochenrinnen der Wa R im Blut besonders im Retroplazentarblut überlegen? *Monatschr f Geburtsh u Gynak* 77 163 1927

303 WEST 106th STREET

## A METHOD FOR SERIAL SECTIONS IN CELLOIDIN\*

BY L. P. FOWLER JR. NEW YORK CITY

IN THE course of a series of experiments in this laboratory requiring histologic sections of petrous bones, it was found that the paraffin methods for serial sections and the usual celloidin methods of staining each section individually were unsatisfactory. To insure a uniform staining reaction and to cut down the time necessary to make a long series of sections the following method was devised.

The apparatus may be quickly improvised in any laboratory. Several ordinary glass slides are prepared by fastening a single loop of 22 gauge brass wire about 2 cm from one end of each. The wire must be flush with the glass on both sides. This is accomplished by bending the wire at right angles in the proper places, so that it will fit the slide, and by twisting the free ends of the wire as tightly as possible without breaking the slide. It was found that if all the wire loops were made on one slide and then slipped on to other somewhat larger slides (slides usually are slightly irregular in width) the loops could be made to fit more snugly and fewer slides were broken. One of these wired slides should be prepared for every section that is to be cut from a single block.

A rack is then made out of 14 gauge copper wire which will hold a number of these slides when they are stacked on top of one another. A rack which holds about 35 to 40 slides is shown in the figure. This size was most convenient to handle but if more sections are desired, a deeper rack can be made and very deep jars used for staining.

The general idea for making the rack can best be ascertained from the figure. The joints are held together with fine wire and need not even be soldered if they are well bound. The tissue to be cut is embedded in the usual way except that the celloidin block is trimmed and mounted rather

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

Received for publication November 4 1928

## PRACTICAL APPLICATIONS

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Routine examination of the milk from the sixth to the ninth day postpartum offers a convenient means of employing the test in maternity practice.

## REFERENCES

1. Bab, H. *Bakteriologie und Biologie der kongenitalen Syphilis*, Ztschr. f. Geburtsh. u. Gynak. 60: 161, 1907.
2. Gucciardi, G. Cited by Bar, P., and Daunay, R., Recherches sur la sero diagnostic de la syphilis chez la femme enceinte et l'enfant nouveau né (méthode de Wassermann), *Obstetrique* 2: 192, 260, 1909.
3. Bruer. Cited by Thomsen, O.
4. Bar, P. and Daunay, R. *Ibid*.
5. Thomsen, O. Wassermann'sche Reaktion mit Milch, *Berl. klin. Wchnschr.* 46: 2052, 1909.
6. Rojas, D. A. La reacción de Wassermann en la leche de mujer, *Semana med.* 26: 100, 1919.
7. Ruser, C. L. Ricerche intorno alla sero diagnosi di Wassermann sul latte muliebre, *Pediatrics* 27: 343, 1919.
8. *Ibid*. Ulteriori osservazioni intorno alla sierodiagnosi di Wassermann sul latte muliebre, *Pediatrics* 29: 121, 1921.

numbers of such sections can be ascertained by counting the spaces either from the bottom or the top of the pile. These numbers should be noted, and the sections gently pulled out placed on other wired slides and put on top of the pile, under the weights.

The whole rack is now run through the desired stains and solutions in tall wide mouthed jars. From the last jar the sections are removed in order from their wired slides and mounted in balsam in the usual way. If the excess celloidin does not conveniently fit under the cover glasses it can easily be cut off.

The mounted sections are now numbered and the sections which slipped out in the alcohol (if any) carefully put into their proper places. The result is a series of sections all equally stained.

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## AN ATTEMPT AT MICROSCOPIC OBSERVATION OF LIVING TISSUE\*

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BY WILLIAM C. CHANEY, M.D. AND LEONARD G. ROWNTREE, M.D.  
ROCHESTER, MINN.

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THIS study of the histology of living tissue was suggested by the work of Krogh on the capillaries and that of Richards on the glomerulus of the kidney. The idea underlying this work was the application of their methods to the study of other living tissue particularly the liver and the spleen.

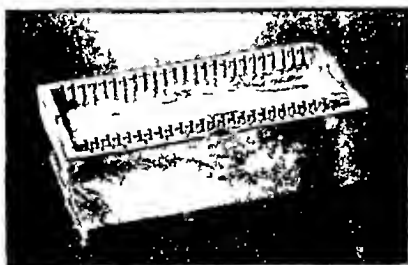


Fig 1—Substage condenser

Investigations began with the spleen. It was realized that a relationship exists between many tissues and that the sharing of function by various organs is incident to the presence in them of a common type of cell. The study of this common type of cell or tissue, the so called reticulo endothelial system claimed primary interest in both the liver and the spleen.

As technical difficulties were overcome and photographs of living cells

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\*From the Division of Medicine The Mayo Clinic and The Mayo Foundation Rochester Minnesota

were obtained, it became evident that a sufficient amount of information could be secured through these methods to make them of practical importance

From the beginning it was evident that there were certain limits to the work which were concerned largely with blood supply distribution of tissues, thickness of tissues, light and heat. A method had to be used which would permit of detailed microscopic study of individual cells such as could be seen with a magnification of almost 1000 diameters and at the same time permit the maintenance of physiologic conditions. This was accomplished by using a substage condenser projecting above the stage of the microscope, preferably fixed within the board on which the animal was held (Fig 1). The tissue to be studied was then placed directly on the condenser and kept moist by

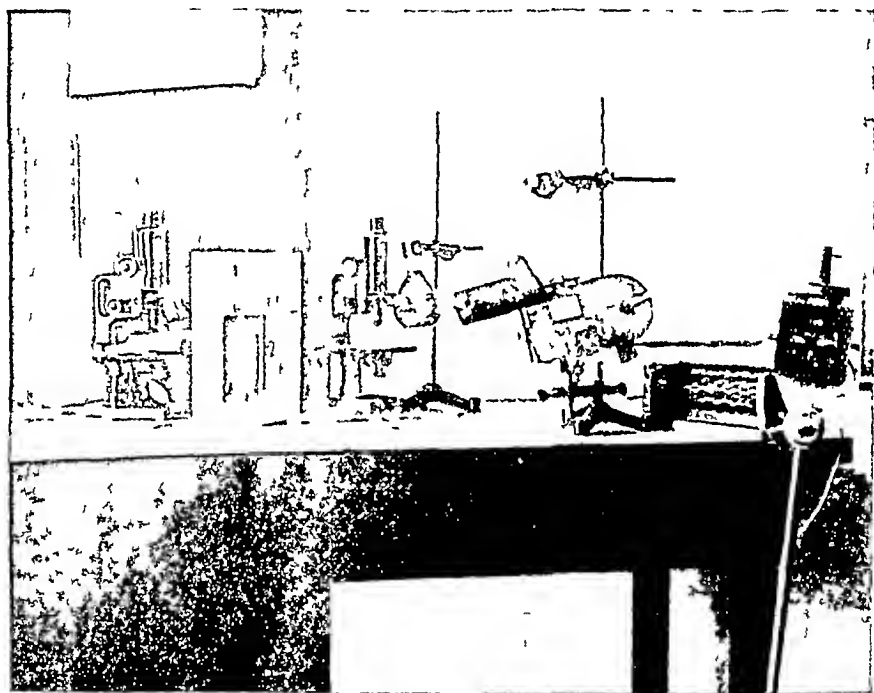


Fig 2—The small arc light is directed toward the jar of alum water. The board on the stage of the microscope contains the condenser. The Florence flask is used whenever reflected light is desired.

the salt solution which filled the moat around the condenser. The body of the animal was brought into close proximity to the condenser to prevent tension on the tissue under study. Very young mice and young frogs were used and found to be much better than older ones.

Intense light was essential to success. This was best supplied by a small arc, the rays from which were passed through a glass jar containing a saturated solution of alum to filter out the heat. For purposes of differentiating the cells, colored solutions such as copper sulphate or potassium dichromate, depending on the tissue under observation were used in the alum solution. Glass filters of various colors were also used (Fig 2).

The use of transmitted light by the method just described, gave the best results, but because of the thickness of certain tissues this method is not

always applicable. Reflected light has been used in a good many instances success depending on placing the rays of light at a point on the tissue such that the angle of incidence is quite acute. An ordinary condenser may be used for this purpose or a 300 cc Florence flask filled with alum solution. There are at least two objections to using reflected light. It shows mostly surface detail and it is difficult to obtain the proper angle of light when the higher magnification is employed such as a 4 mm objective.

Thus far our interest has centered around two problems: the function of the reticular cells in the reticulo endothelial system first described as "macrophages" by Metschnikoff and later by Evans and the relationship existing between the sinuses, capillaries and venules of the spleen.

Effort was made to show the process of phagocytosis by these reticular cells to determine whether they are more active in one organ or another and how they behave toward particulate bodies, cells of other animals, parasites of the blood stream and injured cells of the same animal. As is well known when dialyzed India ink is introduced into the circulation of a frog, it is taken up by the endothelial cells of other tissues. The particles of the ink seem to stick to the endothelial cells very much as though these cells were droplets of honey. The free end of the ink particle may then wave to and fro in the blood stream, may be subjected to constant bumping by erythrocytes, may be dislodged or may finally come to occupy a place within the cytoplasm of the cell. In the frog the liver appeared to take up more of the ink than any other tissue, the spleen being second, then the lungs, then the capillaries of the intestinal tract and finally the kidneys.

In unstained tissue difficulty is of course encountered in differentiating the types of cells. It is evident that the minute cytologic study of living tissue and cells will necessitate the use of vital stains as a means of differentiation. The application of vital dyes even to the surface of an organ is of marked value in the differentiation of cells. The spleen of the white mouse, for example, when viewed without staining appears as a homogeneous structure although here and there a blood stream may be discerned. If, on the other hand, methylene blue is injected into the peritoneal cavity an hour or so prior to the observation, the cells of the spleen will have taken up such an amount of the dye as to make differentiation of cells seem a possibility by this method.

With reflected or transmitted light the blood vessels are usually recognized readily because of the constant movement of the cells. The frog's corpuscles, particularly, are admirably suited for the study of cells in the blood stream because of their size and their large nuclei. Many attempts have been made to examine blood cells after they had been stained with vital dyes and returned to the circulation of the same animal but without significant success since the blood plasma or the liver or some other agent has been effective in the discoloration or the destruction of the cells to a large degree.

Arterial capillaries and veins have been identified in both liver and spleen. It is difficult to decide whether or not splenic sinuses have actually been visualized. In the spleen large channels have been seen. Alteration of function of venous capillaries has been observed in the spleen of the frog corresponding to the phenomenon observed by Krogh in the muscles of frogs.

## AN AUTOMATIC FILTERING DEVICE\*

By MANUEL G. GICHNER, M.D., BALTIMORE, MD

**I**N LABORATORY practice it is often necessary to recover the precipitate from a large volume of fluid upon a small filter paper, as in the preparation of Romanowsky stains or in the preparation of protein antigens by extraction

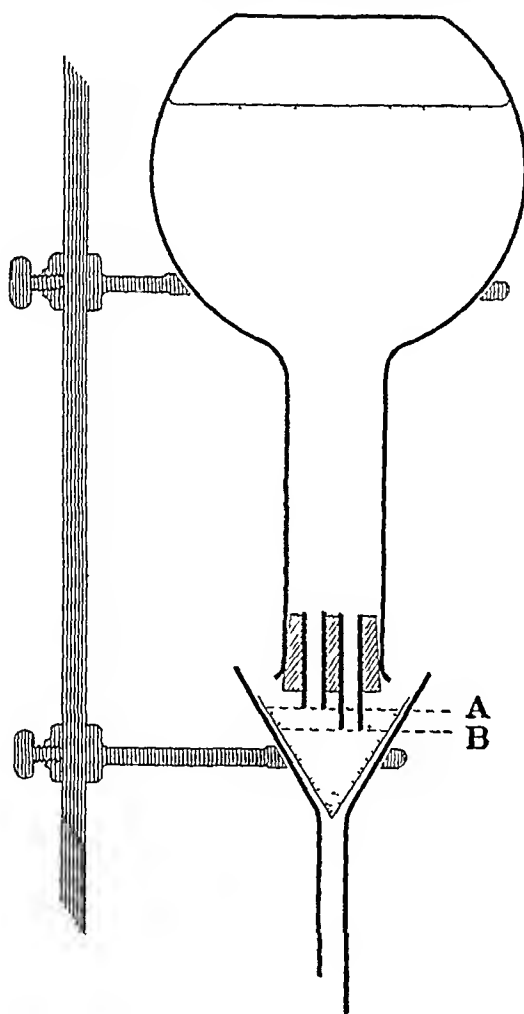


FIG. 1

with dilute sodium hydroxide and precipitation with dilute hydrochloric acid. The procedure frequently necessitates an expenditure of time and attention out of all proportion to the importance of the end accomplished. This apparatus was devised to facilitate such filtrations.

\*Received for publication December 18 1928

The accompanying drawing is self explanatory. Two lengths of glass tubing are inserted into a two hole stopper. The difference in length of the tubes should be slightly greater than the diameter of the lumen, and their inner ends should be flush with the inner surface of the stopper. The stopper is inserted into a flask containing the material to be filtered and the flask is inverted over the filter. Properly adjusted ring supports hold flask and filter in the desired position. Fluid escapes filling the filter to the level of the shorter tube (level A) when no more air can enter the flask and the flow ceases. As filtration proceeds, the level of fluid in the filter falls until a level is reached at which an air gap enters the shorter tube (level B) and the fluid rises to the previous level (level A) when flow again ceases. It is sometimes necessary to secure the stopper to the flask with stout cord, especially if large volumes (five liters or more) are handled at one filtration.

It seems that so simple a device must have been previously described, but I was unable to find it in a cursory survey of the literature. Several very experienced chemical technologists whom I consulted believe it to be a new apparatus.

2426 EUTAW PLACE

## A NEW HEAD HOLDER FOR THE GUINEA PIG BOARD\*

By SUSAN GRIFFITH RAMSDELL M. A. PHILADELPHIA

GUINEA pig boards in general use in most laboratories have been of the simplest improvised sort since the usual catalogue offerings have been of the complicated, multiple purpose type, and therefore prohibitively expensive. The exception has been that devised by Dr. A. F. Coea of the Cornell Medical School, from this model the one herewith presented varies in the substitution of a different type of head holder, the original spring bar for the mouth being replaced by a set of three sliding brackets, adjusted with wing nuts to fit tightly around the jaws thereby holding the head firmly without discomfort (Fig. 1 A and B). It has the further advantage that the animal can be held equally well in the face down position. There is thus provided a board which answers certain definite laboratory needs in an operating board.

- 1 For the greatest possible immobilization of the animal
- 2 For dispensing with the use of an assistant
- 3 For unlimited use in the various technical procedures on this animal
- 4 For an inexpensive, simple various use apparatus

It has been found of particular advantage in the following technical procedures

- 1 Heart puncture where the usual high mortality is due largely to sudden movement of the animal while the needle is in or around the heart
- 2 Jugular puncture where quietness of the animal is even more important for success in entering the vein

Received for publication October 6 1933

From the Research Institute of Cutaneous Medicine Philadelphia



3 Ear vein injection of Rous<sup>1</sup> which is of the greatest use in many instances, but which has been possible hitherto only with the aid of an assistant (Fig 2)

4 Intracutaneous skin-testing, and many other uses which are as readily apparent

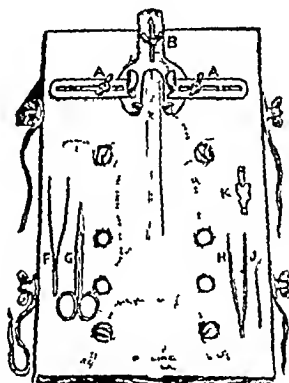


Fig 1—The guinea pig board of Coca with a new type of head-holder



Fig 2—Position for ear vein injection. The left ear has been shaved

In addition, with this device one may carry out the usual laboratory procedures upon white rats without the aid of an assistant

The George P. Pilling and Sons Company, Philadelphia, has been kind enough to carry out my designs

<sup>1</sup>Rous P. J. Exper. Med. 27: 459, 1918

## A MODIFICATION OF THE BELL TYPE OF STETHOSCOPE\*

BY BURGESS GORDON, M D, PHILADELPHIA

A STETHOSCOPE with a metal or hard rubber chest piece may cause discomfort to patients who are nervous and emaciated. If pressure is applied to the instrument which is often necessary when the ribs are prominent the discomfort is considerably increased.

A modification of the bell type of stethoscope (Fig 1) consists of a circular metal base 3.5 cm. in diameter to which is attached a projecting soft rub

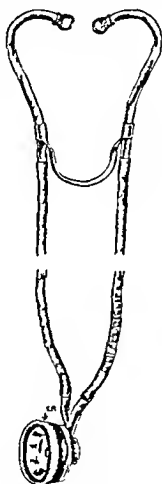


FIG 1

ber ring. On the reverse side the central portion is extended upward to serve as a finger rest and point of attachment for the steel and rubber transmission tubes which protrude at right angles from the chest piece.

The rubber ring is warm, yields to the irregularities of the chest wall, and due to adherent qualities tends to reduce the passage of "hair noises" and other extraneous sounds. The chest piece, owing to shallow construction and the use of side rather than vertical tubes, permits examination without disturbing the position of the patient in bed.

From the Department for Diseases of the Chest, Jefferson Hospital  
Received for publication October 6 1918  
The stethoscope is manufactured by George P. Filling and Son Company

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M D ABSTRACT EDITOR

**CARBON MONOXIDE** A Simple Test for the Recognition of, Schwarzacher, W  
Deutsche Ztschr f d ges gerichtl med 12 510, 1928

In three tubes set up

1 CO blood diluted 1 200 with water

2 Normal blood

3 0.2 per cent cuprous sulphate

Tube 1 is superimposed on tube 3 (blue against a light background) with the control blood at the left (yellow against a light background) If observed so that the control blood is superimposed upon the copper solution it appears green and the CO blood directly to the right of it is red

**GASTRIC ANALYSIS** Minum Method for Analysis of Gastric Content, Einhorn, M  
New England M J 198 941, 1928

The following method permits the qualitative and quantitative analysis of extremely small amounts

The same pipette should be used throughout, the tip being bent at an angle of 60 so that, when held horizontally, the same size drop is always delivered

## Solutions

Yellow Solution 1 1 minim of Topfer's solution to 5 cc of N/100 NaOH

Yellow Solution 2 2 minims of Topfer's solution to 5 cc of N/200 NaOH

Yellow Solution 3 3 minims of Topfer's solution to 8 cc of N/400 NaOH

Scarlet Solution 1 1 minim of phenolphthalein to 12 cc of N/100 NaOH

Scarlet Solution 2 1 minim of phenolphthalein to 7 cc. of N/200 NaOH

Scarlet Solution 3 3 minims of phenolphthalein to 9 cc of N/400 NaOH

For every drop of No 1 solution (yellow and scarlet) the final reading is increased by 10, for every drop of (yellow and scarlet) No 2 solution, the final reading is increased by 5, and for every drop of either No 3 solution the final reading is increased by 2.5

## Method

### Free HCl

Place one clear drop of gastric contents on a porcelain plate or in a paper tray Add drop by drop yellow Solution 1 until a reddish yellow color appears Then change to yellow Solution 2 and 3 and add these successively until the specimen is exactly the same color as the yellow solution

(If this occurs at once the specimen contains no free HCl In such case, the examination of a second drop should be made using yellow Solutions 2 and 3 to measure any small amounts which may be present)

For each drop of Solution 1 add 10, add 5 for each drop of Solution 2, and 2.5 for each drop of Solution 3

Thus using 2 drops of No 1, 1 drop of No 2, and 1 of No 3 the reading is Free acid = 27.5

**Total Acidity** The same technic is followed using the scarlet solutions until the specimen is almost the same color as the solution Using the same computation above gives directly the amount of total acid

# RENAL FUNCTION A New Test Brain E T and Kay H D Quart J Med 1 203 1929

The test described is based upon the excretion of extra phosphorus in the hour following the intravenous injection of 5.0 mg of sodium glycerophosphate. In normal individuals at least 150 mg is excreted. The technique is as follows:

At about 6 A.M. the patient is given a light breakfast devoid of fat in order to prevent as far as possible a lipemia. At 8.30 A.M. he is told to empty the bladder and at 9.30 is given 400 cc of water. At 10.30 A.M. the first (two hour) specimen of urine is collected and measured and a portion is taken for total phosphorus analysis. (At this stage a resting blood sample may be taken if required.) Ten cc of glycerophosphate in approximately 50 per cent sterile solution is then slowly injected into the median basilic vein from a record syringe. The time is now taken. Exactly one hour after the end of the injection the second sample of urine is collected (precautions being taken to empty the bladder completely) and measured. After the proteins have been precipitated with trichloroacetic acid phosphorus determinations (free and total phosphorus) are made in duplicate on both urinary samples a modified Briggs method being used. The normal urinary excretion of phosphorus in milligrams per hour is calculated from the values in the first specimen (8.30 to 10.30 A.M.) and it is assumed that this rate would be maintained approximately in the next hour. By subtracting the hourly normal value from the amount of organic plus inorganic phosphorus in the hour following the injection the "excess phosphorus excretion" is obtained. Comparisons have been made in 44 cases of renal disease between this test and the urea concentration and phenolsulphonephthalein tests. The information provided by the glycerophosphate test has been everywhere at least as valuable as that derived from the other two tests and in certain instances where there has been a divergence between them the glycerophosphate test has agreed most closely with the clinical observations.

# OVA Demonstration of in Feces Garin Doubrow and Mounier Lyon Med 141 341 1928

A modification of Telemann's method for the concentration of helminth eggs is proposed. The new method is rapid and has a very high coefficient. It does not cause shrinkage of the eggs. The procedure is as follows: To 30 cc of hydrochloric acid sulphuric ether is added in small quantities in a graduated flask of 120 cc which is constantly shaken under a stream of cold water. A limpid homogeneous mixture forms at last. The addition of ether is continued the flask being kept continually shaken under the stream of cold water until the flask is filled. The liquid now separates into two layers a lower clear layer and an upper layer which also clarifies rapidly. It is necessary to shake the reagent when used. A portion of feces is emulsified in from 5 to 20 times its volume of water. This is allowed to sediment for a minute to allow the larger particles to sink. About 10 cc are then poured into a centrifuge tube. Three or 4 cc of the reagent is added and the tube is vigorously shaken. When the contents are thoroughly mixed about 30 turns in the centrifuge separate the fluid into three distinct layers. The bulk of the contents of the tube is thrown out and the last 2 or 3 drops which remain behind are examined under a coverslip. The procedure including the examination of the preparation occupies five minutes only. It is exceptional to find vegetable debris but muscle fiber fragments when badly digested may occur in quantity in the preparation.

# GRAM STAIN A Quick Method for Staining Gram Positive Bacteria in Tissues Little R D Arch Path 5 828 1928

Paraffin sections of about 5 microns thickness or frozen sections fixed to the slide by the celloidin method are brought down to water in the usual way.

1 The sections are stained for thirty seconds with ammonium oxalate crystal violet (Crystal violet 2 gm 9.5 per cent alcohol .0 cc 1 per cent ammonium oxalate in distilled water 80 cc. The preparation is filtered after it has dissolved. It keeps well.) For tubercle

and lepra bacilli, staining for a longer period or heating is often necessary. Heating for ninety seconds on a hot plate at from 50 to 52° C has been found satisfactory.

2 The sections are washed in tap water.

3 Lugol's solution I KI H O = 1 2 100 is applied for thirty seconds.

4 The sections are washed in tap water.

5 Acetone (from dropping bottle) is applied until no more color is removed (about ten to fifteen seconds).

6 The sections are washed in water (they should not be allowed to dry after the acetone is applied).

7 They are counterstained with 0.5 per cent safranine in water for thirty seconds.

8 They are washed in water.

9 They are dehydrated and differentiated with acetone. A certain amount of red comes out in this process, leaving the cell nuclei deep red, and the cytoplasm pink.

10 They are cleared with xylol (in moist weather it may be necessary to blot off the xylol once with filter paper as the acetone is quite hygroscopic).

Gram positive bacteria are blue black, gram negative organisms red, cell nuclei deep red, cytoplasm of lymphocytes and plasma cells moderately deep pink, cytoplasm of other cells, pale pink, fibrin, pale pink, collagen, usually pink, sometimes light violet in dense bundles.

#### SPINAL FLUID Effect of Storage (Icebox) On Cell Count In Pathologic Cerebrospinal Fluid, Novick, N Arch Neurol & Psychiatr 21 658, 1929

In a study of the effect of storage on the cell count in fifteen spinal fluids kept at ice box temperature (about 8° C), a general tendency for the cells to diminish gradually from day to day was found. The loss is apparently due to the lytic effect of the spinal fluid itself, as was evidenced by microscopic study.

Twenty four hours' storage at ice box temperature does not invalidate the clinical significance of the cell count.

#### TUBERCLE BACILLUS Culture of, Lichtenstein, C Centralb Bakteriöl 108 239, 1928

Mix 1 or 2 cc of sputum or other material with 10 cc of 10 per cent HCl, shake for ten minutes and then centrifuge for ten minutes. Inoculate the sediment on the medium of Petragram prepared as follows. Mix 150 cc of milk, 6 gm of potato meal and a piece of potato the size of an egg, chopped finely. Place in a boiling water bath and shake for ten minutes, then let stand in the bath for one hour. Cool to 50° and add 4 whole eggs, 1 egg yolk, 12 cc of glycerol, and 10 cc of 2 per cent malachite green. Shake vigorously and filter through gauze. Tube and sterilize at 90° for twenty minutes on three consecutive days.

#### TUMORS Examination of Fluids for Tumor Cells, Zemansky, A P Am J M Sc 175 489, 1928

The technique is a very simple one. The fluid in a large Erlenmeyer flask is allowed to settle by gravity, by standing overnight. The clear upper layer is poured off and as large a quantity of the cloudy sediment as can be conveniently handled is centrifuged in a wide tube (capacity 50 cc), tapering to a point. This is centrifuged at moderate speed for at least twenty minutes. The supernatant fluid is poured off without disturbing the sediment, and the tube is filled with 10 per cent formalin. This is allowed to stand for twenty four hours, when the formalin is poured off. By this time, the sediment is sufficiently hardened to be removed from the tube without falling to pieces. It is gently separated from the tube with a thin blade, lifted out with forceps, and cut from above downward, in order to obtain all layers of the sediment.

This is important, as tumor cells may be missed by cutting only the top or bottom layers. The two halves are then hardened in graded alcohols, run through chloroform paraffin, embedded in paraffin, cut, and stained by hematoxylin and eosin or hematoxylin alone.

**ANTHRAX** New Method for Obtaining High Titer Diagnostic Precipitin Sera, Rosenberg, B and Romanow D Centralbl Bakteriell 110 102, 1929

Wash off a ten to twelve hour agar growth at 33 to 34 in 10 cc of normal saline Add 100 cc of distilled water 20 cc of saturated sodium chloride solution, and 6 to 7 drops of acetic acid 50 per cent Heat in the Arnold twenty to thirty minutes Filter, wash coagulum in alcohol and dry Grind in a mortar with 2 cc of normal saline, let stand about ten minutes and inject intravenously

**SPIROCHETES** Method for Culture of *Sp. iterohaemorrhagiae* Fukushima B Sc Rept Gov Inst Infect Dis Tokyo 6 545 10-7

Citric acid	0.1
Calcium chlorate	0.1
Sodium hydate	55
Tap water	1000 cc

This mixture is sterilized for one hour at 100 C 0.3 gm saccharose is then dissolved in a little distilled water, heated for ten minutes to 100 C and added to the above solution Eighty cc of the resulting medium are then mixed with 20 cc of inactivated horse serum, the resulting  $P_H$  being 7.4 to 7.6 the mixture is then run into test tubes 10 cc. in each

Citric acid	0.1
Calcium chloride	0.1
Sodium hydate	55
Glucose	0.2
Tap water	1000 cc

After sterilization for one hour at 100 C 80 cc of this solution are mixed with 20 cc of inactivated horse serum and divided into test tubes as in the case of the first medium

The tubes containing the media are heated for one hour at 36 C and then one drop of infected guinea pig blood is added to each tube they are then incubated at 37 C for forty eight hours For making subcultures one drop of normal guinea pig blood is added to each tube and then 0.1 cc of the culture For making plate cultures the first medium is used, but 75 cc of the mixture is mixed with 25 cc of horse serum After heating to 36 C for one hour one drop of normal guinea pig blood is added to each tube and these left for twenty four hours for the fibrin to precipitate The clear supernatant fluid is then poured on agar plates (without bouillon or peptone) in the proportion of about 1 to 5 The culture of spirochetes preferably after two or three passages is then sown on the surface of the plate which is then grown for four days at 37 C and after ward at 30 C

**TUBERCLE BACILLUS** A New Stain For Ransom C G J Tenn Med Assn 21 10 1029

100 cc of distilled water

10 cc of N/10 NaOH or 1 cc of N/1 NaOH

4 per cent or 4 gm of safranine

Dissolve well

The stain is applied and steamed for two minutes

**AMEBAE** Stained Preparations in the Study of Amebic Infections Riley W A Minn Med 12 65, 1929

The following technic is advocated

Schandann's fluid

Saturated aqueous solution of corrosive sublimate	60 cc
95 per cent ethyl alcohol	70 cc
Glacial acetic acid	3 cc

It should be heated until steam is given off (60° to 70° C) immediately before using. Handling slides with forceps or allowing metal to come in contact with the solution in any way must be avoided or precipitates will ruin the preparations.

#### Technic

1 By use of an applicator stick, or a match, smear the fresh fecal material over two thirds of a thoroughly clean slide. The smear should be thicker than for ordinary bacteriologic work and must not be allowed to dry at any stage until finally mounted in balsam.

2 Fix in the warm Schaudinn's fluid fifteen minutes.

3 Rinse in 50 per cent alcohol three to five minutes.

4 Transfer to 70 per cent alcohol to which has been added enough tincture of iodine to give a bright straw color, ten minutes. If the fluid is bleached renew it.

5 Harden in 95 per cent alcohol, one hour or more.

6 70 per cent alcohol, five minutes.

7 50 per cent alcohol, five minutes.

8 Rinse in distilled water.

9 Mordant in a 4 per cent solution of clear violet crystals of iron alum (ammonio ferric sulphate) in distilled water, six hours.

10 Rinse in water.

11 Stain in 0.5 per cent ripened solution of hematoxylin in distilled water, six hours to overnight.

12 Rinse in water.

13 Differentiate in 2 per cent iron alum solution, controlling by rinsing in water and examining under the microscope from time to time. The background should be grayish and it is desirable to differentiate the slides to a slightly different degree, since it is not feasible to search long for cysts as controls.

14 Wash in running water, twenty minutes.

15 Dehydrate in 50, 70, 95 per cent and absolute alcohol, five minutes each.

16 Clear in xylol, five minutes.

17 Mount in Canada balsam.

The periods suggested for mordanting and staining are not fixed, but usually work out conveniently in practice and give excellent results. The whole process may be greatly reduced, even to the extent of the mordanting for a half hour and staining for an hour, but this short method is more difficult to control.

The properly prepared slide is not only valuable for immediate diagnostic purposes, but affords opportunity for careful, unhurried study. Moreover, it constitutes an important part of the permanent record of the case.

#### BLOOD FAT Demonstration of, in Osteomyelitis, Nakata, M. Deutsche Ztschr f. Chir. 213: 132, 1928.

Place 0.5 to 1 cc of blood from a vein in an alcoholic solution of Sudan III and examine microscopically after five minutes.

## REVIEWS

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Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building  
Richmond, Va

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### *Technique of Contraception\**

A FULL presentation of the subject by the director of the Clinical Research Department of the American Birth Control League

The first section is devoted to quotations representing the expression of opinion of prominent persons such as Havelock Ellis William A Pusey Lord Dawson of Penn Sir Arbuthnot Lane Prince A Morrow Abraham Jacob Jos B DeLee, Adolphus Knopf Charles E De M Sajou Clarence C Little Raymond Pearl and others including members of the clergy

There follows a chapter on the medical indications for contraception the physiology of impregnation and then a section devoted to a critical clinical study of all the contraceptives that have been recommended The writer next describes the results of comparative studies made under the direction of the Birth Control League and presents his conclusions concerning the most efficient contraceptive

The subject is treated judiciously but thoroughly

The second portion of the book deals with the accomplishment of the social agencies that are interested in eugenes birth control and similar aspects of the general subject The last chapter presents rather briefly the present legal status of contraception in the different states

The book is sold only to physicians and may be recommended as being authoritative and informative

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### *Textbook of Clinical Neurology†*

ALL good books on the same medical subjects cover essentially the same field Their differences lie in their manner of presentation and interpretation and depending upon these factors, one or another of them is especially appropriate for this or that person whose interest in the subject is from this or that angle or viewpoint

Neustadter has written his work primarily for the medical man who needs a reference volume when he runs across neurologic problems The logical basis for the author's presentation and one in which it differs from most other volumes on neurology is then first his classification in a general way under broad objective findings such as the spastic paralysis the flaccid paralysis the ataxias tremors and spasms the trophic disorders the vasomotor disorders and functional neuroses and second his presentation under each individual subject of symptomatology prior to other discussion Thus the practitioner finding

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*Technique of Contraception The Principles and Practice of Anti Conceptional Methods* By James F Cooper M.D Medical Director of the Clinical Research Dept of the American Birth Control League Formerly Professor of Clinical Surgery Fochow Union Medical College Fochow China and Clinical Instructor in Obstetric Boston University Medical School Cloth 71 pages Day Nichols Publishers New York N Y

*Textbook of Clinical Neurology for Students and Practitioners* By M. Neustadter M.D Ph.D Introduction by Edward D Fisher M.D 8 illustrations some in colors 60 pages F. A. Davis Co Philadelphia 1929

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NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume

We trust that the scientific information printed in the e pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto



a general type of neurologic lesion may first go through that subject to see whether the symptomatology and gross findings correspond to those of his case, and when he finds the appropriate one or more examples he may then study these in greater detail.

The first ninety pages describe in all necessary detail methods of neurologic examination. The work is unusually well illustrated. The volume should find a welcome place on the reference shelves of internists, and clinicians.

### *Stedman's Medical Dictionary<sup>1</sup>*

A STANDARD medical dictionary brought strictly up to date. We do not envy the lexicographer who must keep abreast of all of the new terms offered for adoption in the medical sciences. His reading must be wide, his understanding deep and his discrimination good.

This volume may be recommended as a standard reference dictionary.

### *Practical Clinical Laboratory Diagnosis<sup>2</sup>*

THIS, the third edition of a well known and deservedly popular book, has been so extensively rewritten and revised as to be largely a new text.

As is well known it is not intended primarily for the laboratory worker, but for the clinician who does his own laboratory work. As a guide to what should be done and how to do it, it well fulfills its purpose.

The reader is not confused with a multiplicity of methods but directed in the use of those considered most suitable in the light of the author's experience.

The table of leucocytic reactions in disease, that concerned with bacteria, should prove useful to clinicians.

The book is profusely illustrated with microphotographs, drawings, and colored plates of extremely practical type.

One criticism may be made, in this reviewer's estimation. The book is obviously intended for the use of clinicians unskilled in laboratory procedures. To entrust to unskilled hands the serologic study and diagnosis of syphilis by means of a single test, a precipitation reaction, no matter how simple or how excellent in the hands of trained serologic workers, seems ill advised, at least, until clinicians are more generally and thoroughly familiar with the pitfalls, complexities, and the inherent vagaries of the serology of syphilis than they are at present.

### *Handbook of Bacteriology<sup>3</sup>*

THIS is the second edition of a well written text intended especially for the use of students and practitioners of medicine.

It has been extensively revised and rewritten in order to conform to the information concerning this subject which has accumulated in the four years since it was first published.

It reflects very ably the modern outlook upon bacteriology and is in every way suited for the purpose for which it is intended.

<sup>1</sup>A Practical Medical Dictionary. By Thomas Lathrop Stedman A.M. M.D. Editor of *Twentieth Century Practice of Medicine* and of the *Reference Handbook of the Medical Sciences*. Formerly Editor of the *Medical Record*. Tenth Revised Edition. Illustrated. 1194 pages. Flexible Fabricoid. William Wood & Company, New York.

<sup>2</sup>Practical Clinical Laboratory Diagnosis. By Charles C. Bass, Dean Medical Department Tulane University and Foster M. Johns, Assistant Professor of Medicine Tulane University. Cloth. 187 pages. 139 illustrations and 6 color plates. Williams and Wilkins Co., Baltimore, Md.

<sup>3</sup>Handbook of Bacteriology. By Joseph W. Bigger, Professor of Bacteriology and Preventive Medicine University of Dublin. Ed. 2. Cloth. 82 illustrations. 452 pages. William Wood & Co., New York.

### *Report of the International Conference on Cancer\**

THIS volume, representing as it does the opinions of an exceedingly catholic group of international experts may be said to represent a cross section of the facts concerning cancer now accepted and also concerning those still under investigation

It well repays a careful reading by the clinician as well as by the special worker

The section on the etiology of cancer, while summarizing such facts as are known and adding others, as yet cannot present a solution to the problem

The section on the relative values of surgery and radiation in the treatment of cancer is of great interest. The views of the enthusiast, the pessimist, and the conservative are all represented with those of the conservative carrying the day

Of equal interest is the section on treatment by chemotherapy with special reference to the use of lead

The cumulative experience thus gathered leads to the inevitable conclusion that the lead treatment sponsored by Blair Bell is without specific effect on malignant neoplasms

In the section on pathology there is a wealth of information. This is true also of the section on diagnosis in which among other things various proposed serologic methods are discussed

A section on radiology, one on the geographic and racial prevalence of cancer and one on public action with regard to cancer conclude a volume of great interest to all who are interested, and who is not? in this most important subject

### *Experimental Bacteriology and Infectious Diseases With Especial Reference to Immunity†*

THIS is a new edition of the well known, standard German work which has already been translated into twelve other languages. It is a treat to turn the pages and look at the marvelous illustrations: 118 colored plates and 200 black and white text figures, many of the illustrations of an excellence found only in German works

Extensive changes and additions have been made in this edition to include the advances made during the seven years since the previous edition, some of the topics most extensively revised and enlarged being the bacteriophage, tularemia, streptococci in scarlet fever, and the question of herpes and postvaccinal encephalitis. However, the size of the two volumes has not been increased as the authors have been able to throw out an almost equal volume of material

Americans have always wondered how the Germans find time to read such elaborate works and the authors in the preface bewail the fact that members of the younger generation have not been as anxious to thoroughly ground themselves in their subject by industriously studying the great works as was previously the case and state that the old spirit must return if the reputation of their professional men is not to suffer

They also state that the difficulty experienced by authors since the war in being unable to obtain foreign periodicals, with the result that their presentations have not been well rounded has now been largely overcome

Report of The International Conference on Cancer. London 1928. Cloth. P88 pages.  
Wm Wood & Co. New York

†Experimental Bacteriology and Infectious Diseases With Especial Reference to Immunity.  
By Dr W Koelle and Dr H Hetsch. Urban and Schwarzenberg. Berlin and Vienna 1929

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Richmond, Va

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## EDITORIALS

### Geographic Variations in the Size of the Red Blood Cell

FIVE million red blood cells are usually taken as the average count of the normal adult male. The count is higher at high altitudes and among certain peoples such as the Scandinavians. Relatively few careful studies have been made to determine variations in the number of erythrocytes in different parts of the United States. Emerson<sup>1</sup> found the mean count of 176 male medical students between the ages of twenty and twenty-five in Baltimore to be exactly 5.0 millions per cmm. The average of 40 normal men from eighteen to fifty years of age in Kansas City was 4.97 millions.<sup>2</sup> Recently comprehensive studies from different parts of this country show widely varying results. In Portland (Oregon), Osgood<sup>3</sup> examined the blood of 137 normal young men between the ages of nineteen and thirty and found the average red cell count 5.39 millions. Wintrobe and Miller<sup>4</sup> report from New Orleans an average count of 5.85 millions in 100 medical students between the ages of nineteen and thirty.

The studies from Portland, New Orleans, and Kansas City also record the relative volume of packed cells after centrifugation. If corrections are made for differences in technique, the average cell volume is everywhere the same, within the limits of error of determination. The comparative data for normal men between the ages of nineteen and thirty has been summarized by Wintrobe and Miller.<sup>4</sup> All observers find approximately 46.5 cc of packed cells per 100 cc of blood. Likewise the amount of hemoglobin per 100 cc as determined by Van Slyke's method is practically the same. Thus in Portland the figure is 15.76 gm per 100 cc, in New Orleans, 15.87 gm, and in Kansas City 15.83 gm. It is apparent the only geographic difference in the erythrocytes is the number, since the total volume of cells and the total hemoglobin is everywhere the same.

This variation in number of red cells is most important since it affects the color and volume index and the average individual cell volume and hemoglobin content. For the indices one must take as normal the hemoglobin in grams and the cell volume in cc per 100 cc of blood per 5 million cells ("hemoglobin coefficient" and "volume coefficient" of Wintrobe and Miller). It is apparent these coefficients vary directly with the cell count since the hemoglobin content and the total cell volume are constant. In New Orleans the hemoglobin coefficient is 13.65 gm and the volume coefficient is 39.7 cc, in Portland, 14.66 gm and 42.2 cc, and in Kansas City 15.57 gm and 45.8 cc. This variation necessitates the determination in every place of the average red cell count and hemoglobin and volume coefficient for accurate calculation of the color and volume indices. Tables such as those given by Osgood<sup>5</sup> can be used only where the red cell count is the same as that found in Portland.

The most desirable measure of volume of the red cell is cubic microns. This of course also varies with the cell count when the total volume of cells remains constant. In Portland the average individual cell volume is 84.4 cubic microns, in New Orleans, 79.4 and in Kansas City 91.6. Similarly the actual hemoglobin content of the average red cell in Portland is 29.2 by  $10^{12}$  gm, in New Orleans, 27.6 by  $10^{12}$  gm and in Kansas City 31.1 by  $10^{12}$  gm. So far no studies of red cell diameters have been reported to compare with other cell measurement. In Kansas City<sup>6</sup> the average red cell diameter in normal adults is the same as that found in Boston and generally accepted as the average cell diameter (7.7 microns).

Emmons<sup>7</sup> has recently reported a study of the interrelation of number, volume, diameter, and area of erythrocytes in man, the dog, the rabbit, the cat and the goat. The red cell count of the goat is 15 millions and of the cat 90 millions per cmm, yet the volume percentage of red cells is little different from that of man. The individual cell volume of the goat is 24.6 cubic microns. The cell thickness, however, is much the same as that of man with almost four times the cell volume. The variation in volume is due almost entirely to difference in diameters. Likewise the total cell surface area in square centimeters per cmm of blood is almost exactly the same in all animals studied. Emmons makes the interesting suggestion that the blood count of any mammal can be determined from data concerning the cell diameter alone.

The observations of Emmons in animals are of great interest in connection

tinuous bleeding from benign lesions of the uterus and rectum, one a case of gastric carcinoma, and in one the anemia was apparently due to syphilis

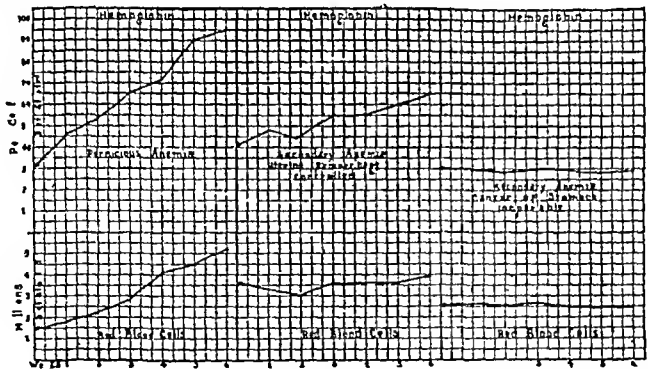
In these groups there were striking differences in the response to treatment In the pernicious anemias the rise in hemoglobin and red cells was uniform, rapid, and steady In the secondary anemias the rise was but slight in the cases in which operation had removed the cause before the treatment was begun, and in the case of gastric carcinoma no change at all was seen

His findings are best seen in the table and chart below

BLOOD CHANGES IN TEN CASES OF PERNICIOUS ANEMIA AND FOUR CASES OF SECONDARY ANEMIA TREATED BY THE MINOT-MURPHY DIET

CASE	HEMOGLOBIN PERCENTAGE		RED BLOOD CELLS	
	ADMISSION	DISCHARGE	ADMISSION	DISCHARGE
Pernicious Anemia	1-----	26 93	1,280,000	5,000,000
	2-----	32 95	1,380,000	5,200,000
	3-----	38 100	1,740,000	5,330,000
	4-----	30 100	1,360,000	5,120,000
	5-----	34 100	1,250,000	5,290,000
	6-----	45 80	1,960,000	3,700,000
	7-----	81 111	3,710,000	5,010,000
	8-----	36 85	3,340,000	4,540,000
	9-----	40 98	1,330,000	4,390,000
	10*	24 65	1,000,000	3,270,000
Secondary Anemia	1-----	43 62	3,270,000	3,990,000
	2-----	48 65	3,000,000	3,750,000
	3-----	20 33	2,080,000	2,660,000
	4-----	28 28	2,580,000	2,460,000

\*This patient remained in the hospital only thirteen days



In view of these results Brill suggests that it is not at all unreasonable to assume an etiologic relationship between the disease and the diet from which it would follow that it would be comparatively useless in the treatment of anemias of secondary origin

To this assumption added weight is brought by Faher<sup>s</sup> of the usefulness of liver treatment as a means of identifying the nature of anemias in infancy and childhood

In three cases in infants, the blood picture in all resembling that of primary anemia, striking and rapid improvement followed the administration of liver extract in two, the third showing no response of any sort but responding promptly to transfusion

The essential difference, as already stated, seems to be that in secondary anemia there is such fatigue of the *hematopoietic* system that blood regeneration is imperfect and delayed though all the requisites are present, while in pernicious anemia there is a lack of erythrocyte elements capable of undergoing maturation.

At all events the common assumption of the past half century that pernicious anemia is a process primarily hemolytic in character must be revised, and we now regard the disease as dependent not upon blood destruction, but upon inadequate blood formation.

The mechanism involved in the response to liver therapy in pernicious anemia has been carefully studied and reported upon by Minot, Murphy and Stetson\* as follows:

Both the percentage and absolute numbers of reticulocytes in the peripheral blood at the peak of their rise are related to the level of the red blood corpuscles at the time treatment is begun. These relations may be employed to help determine the potency of material fed a patient.

Up to the peak of the reticulocyte rise the increase in the concentration of total red blood corpuscles may be ascribed almost entirely to the production of reticulocytes when the red blood cells are less than 2.8 million per c mm.

In cases with over 3 million red blood cells per c mm, or in cases which have received daily maximal amounts of potent extract for over twelve days the increase in the total concentration of red blood cells is dependent chiefly on the liberation from the bone marrow of mature corpuscles.

In pernicious anemia with less than 3 million red blood cells per c mm the absence of a reticulocyte response renders it exceedingly probable that an impotent extract has been employed. On the other hand if the extract given is known to be potent and no distinct reticulocyte increase follows it is very improbable that the patient has pernicious anemia provided his red blood cells are below 3 million per c mm.

The number of reticulocytes that appear in the blood at the peak of their rise is roughly proportional to the amount of active principle ingested daily, provided submaximal amounts are fed to patients with less than 2.5 million red blood cells per c mm. When large amounts are given the reticulocytes are apt to appear sooner and vary more in concentration in different cases with the same erythrocyte level.

In some cases there are probably many primitive cells to be transformed within the marrow and in others relatively few, so that in the latter more reticulocytes would not enter the blood stream even if more active principle were given. Standardized extract from 500 or 600 gm. of liver apparently is sufficient to produce a maximum response of young red blood cells in essentially all cases.

There is a direct relationship between the rate at which the red blood corpuscles increase and the amount of potent material fed, up to a maximum. On the average when liver extract derived from 500 to 600 gm. of liver has been fed daily to patients with less than 2 million red blood cells per c mm of blood it has increased the concentration of these cells about 2.5 million per c mm in thirty days. After two months of adequate therapy with liver

reticulated blood cell counts in the vicinity of 5 million per c mm are to be expected

The active principle effective in pernicious anemia appears particularly to stimulate the formation of red blood cells. The return to normal of red blood corpuscles usually proceeds more rapidly than that of hemoglobin as a result of treatment with liver, with kidney, or with liver extract.

The hemoglobin increases perhaps more slowly in cases treated with liver extract and a diet poor in sources of iron, and in foods that accelerate hemoglobin regeneration, than when potent extracts are given and the diet is balanced, and rich in such foods.

It is emphasized that the continued ingestion of some source of the active principle effective in pernicious anemia is necessary to prevent relapse in disease.

As stated by Means and Richardson, the accumulating facts of the newer types of pernicious anemia warrant these conclusions:

"1 Pernicious anemia is a chronic disease of unknown etiology, the chief manifestations of which can be held in abeyance as long as adequate amounts of specific substance contained abundantly in liver and kidney are received.

"2 The supply of this substance can be rendered adequate in persons suffering from the disease by feeding large quantities of natural foods which contain it (liver or kidney), or active fractions thereof or perhaps also through the provision of the means for natural gastric digestion.

"3 Shortage of the specific substance is clearly responsible for the abnormalities in the liver and blood. The cause of the shortage is not completely known. Castle's work suggests that the gastric defect may play an important rôle.

"4 Whether the normal human being requires any extraneous supply of the specific substance or can synthesize what he needs is not yet known. The problem of whether the active substance now found is in the state that the body needs or is merely a necessary building stone remains unsolved."

## REFERENCES

- Wotch, G. R., and Murphy, W. P. Treatment of Pernicious Anemia by a Special Diet, *J. A. M. A.* 87: 471, 1926.
- Hipple, G. H., and Rabscheidt Robbins, F. S. Blood Regeneration in Severe Anemia, *J. Physiol.* 72: 408, 1925, *Ibid* 72: 419, 1925.
- Means, J. H., and Richardson, W. Impressions of Nature of Pernicious Anemia in Light of Newer Knowledge, *J. A. M. A.* 91: 923, 1928.
- Castle, W. B., and Locke, E. A. Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia, Read Before the Am. Soc. for Clin. Invest., April, 1928.
- Jessler, K. K., and Maurer, S. Treatment of Pernicious Anemia With a High Caloric Diet Rich in Vitamins, *J. A. M. A.* 89: 768, 1927.
- Jessler, K. K., Maurer, S., and Laughlin, R. The Relation of Anemia, Primary and Secondary, to Vitamin A Deficiency, *J. A. M. A.* 87: 476, 1926.
- Wall, I. C. The Specificity of the Minot-Murphy Diet in Pernicious Anemia, *J. A. M. A.* 89: 1215, 1927.
- Ther, H. K. Value of Liver Extract (343) in Identifying and in Treating Certain Anemias of Infancy and Childhood, *Am. J. Dis. Child.* 36: No. 6, 1121, 1928.
- Wotch, G. R., Murphy, W. P., and Stetson, R. P. The Responses of the Reticulocytes to Liver Therapy Particularly in Pernicious Anemia, *Am. J. Med. Sc.* 175: No. 5, 581, 1928.

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## Side Lights of the Convention

The Eighth Annual Convention of the American Society of Clinical Pathologists was held in Portland just previous to the meeting of the American Medical Association and again registered a very successful meeting.

There was a cordial reunion of all the old war horses of the early days together with many enthusiastic recent members. The informal chats in the lobbies between the scientific sessions or at meals formed a very delightful feature of the proceedings. Every visitor vowed that nothing would prevent him from attending next year's convention. Many of the members were accompanied by their wives. There was also a good sprinkling of lady pathologists who participated in the proceedings.

All the past presidents with two exceptions were among those present. It was an inspiring sight to see them sitting at the speaker's table on the evening of the banquet.

No inconsiderable credit for the success of the convention is due to the wonderful preparations made by the Local Committee especially Drs Foskett and Manlove which insured us smooth functioning of the machinery. Nothing had been left undone. The entertainment of the members was one that will long be remembered by those who participated in the automobile ride on the Columbia Highway and around Mount Hood. The pleasant incidents of the trip will be the topic of conversation by the winter fireside especially among those who took snapshots of the scenic wonders encountered.

The visiting ladies were entertained by a drive around the city and a bridge luncheon at the home of Mrs H H Foskett wife of our Chairman of the Committee on Arrangements.

A number of the members en route to or from the convention took advantage of the opportunity to visit the Office of the Society at Denver and were entertained by the local pathologists. Among those who registered were Dr and Mrs I H Black of Dallas Texas Dr M D Bull and Dr J E Robinson also of Dallas Dr G B Kramer Youngstown Ohio and Dr F B Johnson of Charleston South Carolina.



The scientific program was all that could be desired and constituted a treat for the members and visiting scientists. The symposium on Undulant Fever elicited considerable discussion.

The Ward Burdick Prize for the best work in research was awarded to Dr. Walter M. Simpson of Dayton, Ohio, for his pioneer investigations in Tularemia. The American Medical Association had presented Dr. Simpson with a prize for research along the same lines a year ago. It was the unanimous resolve of the Committee on Research of the American Society of Clinical Pathologists that the first medal be given to Dr. Simpson. The medal was presented to the recipient by the President, Dr. F. W. Hartman, who in a few well-chosen remarks complimented him on his labors. Dr. Simpson was the most surprised man in the audience and though usually fluent and ready in speech was too stunned to reply except to express his thanks. The presentation of this medal will undoubtedly act as a stimulus to our Fellows to emulate the example of Dr. Simpson.

The Round Table Discussion devoted to economic matters evoked the usual lively and spirited discussion permitting the members to let off high pressure emotion and get grouches out of their system in the course of their jeremiads on some deplorable phases of the practice of clinical pathology. This year, however, was marked by a greater optimism and prospects for improvement in conditions.

The business session was marked by fine harmony and unanimity. The following oficers were elected:

President Elect, Dr. Kenneth M. Lynch, Charleston, South Carolina

Vice President, Dr. H. H. Foskett, Portland, Oregon

Members of the Executive Committee

Dr. F. W. Hartman, Detroit, Michigan

Dr. A. S. Giordano, South Bend, Indiana

Members of the Board of Censors

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Dr. Alvin G. Foord, Buffalo, New York

Dr. C. Y. White, Philadelphia, Pennsylvania

Dr. E. S. Maxwell, Lexington, Kentucky

Dr. Wm. Thalheimer, Chicago, Illinois

Considerable scrutiny was made of the applications of the new members, each one having been carefully gone over and investigated by the Board of Censors and considered in the executive session. The following clinical pathologists were elected to membership:

Fellows

Dr. Arthur Amolsch, Detroit, Michigan

Dr. O. A. Brines, Detroit, Michigan

Dr. Henry T. Brooks, New York City, New York

Dr. Frank L. Kelly, Philadelphia, Pennsylvania

Dr. Frank P. Hunter, Lafayette, Indiana

Dr. Theodore Helmbold, Pittsburgh, Pennsylvania

Dr. Walter G. Bain, Springfield, Illinois

Dr. Hugh G. Jeter, Oklahoma City, Oklahoma

Dr. Harry Langdon, Indianapolis, Indiana

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Reinstatement

Dr. W. W. Hall, Mercy Hospital, Watertown, New York

Associate Membership

Dr. Willa M. F. Davis, Washington, D. C.

Corresponding Membership

Secretary of the British Pathologists Association

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## CLINICAL AND EXPERIMENTAL

### XANTHEMIA AND XANTHOSIS (CAROTINEMIA) A CLINICAL STUDY\*

BY WILLIAM C. BOECK, PH.D. M.D. ROCHESTER, MINNESOTA,  
AND WALLACE M. YATLER, M.D. † WASHINGTON, D. C.

A DIET composed of much fruit vegetables butter or eggs foods rich in yellow lipochrome pigments may result in the development of a canary yellow, or light orange pigmentation in the palms soles, nasolabial folds, and sometimes, but to less intensity, in the skin generally. Von Noorden, in 1904 called attention to this condition among some of his diabetic patients and, in 1907, his observations were published. He thought it to be a manifestation of diabetes and named it "xanthosis diabetica." Following this announcement other investigators recorded similar observations among diabetic patients. Moro, in 1908, discovered that the same type of pigmentation 'pseudoicterus,' developed when carrots were added to the diet of some normal infants. This observation was recorded by other investigators later for both children and adults who did not have diabetes.

Salomon, in 1919, identified the pseudoicterus of nondiabetic patients as identical with the xanthosis diabetica of von Noorden. He and others showed that the pigmentation was of exogenous origin, and was derived by the absorption of the lipochrome pigments, carotin and xanthophyll present in the fruit vegetables butter, and eggs of the diet. It disappeared in time when such foods were removed from the diet. Salomon pointed out that probably the first case of this nature, which he termed "xanthosis," was described by Hayem in 1897, as occurring in an old dyspeptic patient. But according to Hashimoto, in 1922, Baeltz in 1896, was the first to describe the pigmentation in persons

\*Work done in the Division of Medicine The Mayo Clinic.

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†Work done while a fellow in Medicine The Mayo Foundation

who had been eating many oranges for a long time Van den Bergh and Snapper, in 1913, showed that there were lipochrome pigments circulating in the blood of the diabetic patients who had the yellowish discoloration of the palms, soles, and nasolabial folds Spectroscopically it was found to be identical with pigments of carrots and other vegetables, chiefly carotin and xanthophyll

Salomon, in 1919, also showed that these pigments were present not only in the blood of persons with the yellowish pigmentation of the skin, but in other persons who did not have discoloration of the skin The circulation of the pigments in the blood he termed "xanthemia," and the condition in which the pigmentation occurred in the palms, soles, and nasolabial folds, "xanthosis," as von Noorden (1904) had done earlier He found that xanthemia might occur without xanthosis but not vice versa

Hess and Myers, in 1919, assigned the term "carotinemia" to those cases in which the yellowish discoloration of the skin occurred following the ingestion of a large quantity of vegetables over a long time They adopted this term because they had failed to find any reference to this condition in the literature However, the work of Moro, in 1908, and of Miura, in 1917, who had referred to the condition as "carotinosis," and the later work of Salomon, in 1919, had been overlooked In view of this fact we believe the terms xanthosis and xanthemia have priority of usage, and that, also, they more accurately describe the conditions Carotinemia literally means the presence of pigment circulating in the blood stream and does not imply the deposition of the pigment in the skin, as these authors intended Investigations have shown that, although the chief yellow lipochrome pigment present in the blood serum is carotin, it is always associated with xanthophyll On the other hand, Palmer, in 1922, cited a case of xanthosis in a patient with diabetes, whose blood serum, when analyzed for lipochrome pigments, contained mostly xanthophyll and but little carotin His diet had been rich in xanthophyllie substances (eggs and green beans) The term xanthemia\* is therefore more appropriate, and xanthosis should be used to signify the visible presence of these yellow pigments in the skin We shall follow this usage of terms A complete bibliography on this subject may be had by consulting the papers of van den Bergh and Muller, in 1920, Palmer, in 1922, Greene and Blackford, in 1926, and Stone, in 1928

Our investigations involved the study of 100 patients with diabetes, 53 patients with other diseases than diabetes, and 36 cases in which there was a diagnosis of xanthosis whether it occurred independently or associated with some disease

We desired to ascertain in these patients the extent of xanthemia and xanthosis and to correlate these two conditions with such factors as sex, age, diet, disease, icterus-index and the serum-bilirubin content of the blood We also made blood fat determinations in a small number of cases to see if there might be any disturbance of the fat metabolism of the body which might explain why xanthosis and xanthemia occurred in some patients and not in others

\*Strictly speaking the term xanthemia implies the presence of all yellow pigments circulating in the blood but we wish to adhere to its original definition as stated by Salomon which limits the term to the lipochrome pigments thereby excluding the bile pigments

## LABORATORY METHODS

A qualitative test for the determination of the lipochrome pigments in the blood was carried out one or more times for each case a quantitative determination also was made in some of the cases. The specimens of blood were collected in the morning between eight and nine o'clock. The patients went without breakfast until after the collection was made.

Qualitatively the presence of lipochrome pigments was demonstrated in the blood serum by adding 2 c.c. of alcohol and 2 c.c. of petroleum ether to 2 c.c. of the blood serum and then centrifuging the mixture (Greene and Blackford). The yellow pigment because of its solubility passes into the upper layer of petroleum ether while the lower layer of precipitated proteins appears colorless. The intensity of the yellow in the petroleum ether varied according to the amount of lipochrome pigments in the blood. In some cases the color was but faintly yellow and in others moderately or markedly yellow.

In most cases the serum bilirubin and icterus index were determined at the same time that the qualitative test for the lipochrome pigments was made. In the course of the investigation cases were found in which the serum bilirubin was normal the icterus index relatively high, and the test for carotin pigment was positive. In these cases the excess of pigment in the blood serum which accounted for the high icterus index was assumed to be due to the lipochrome pigments a point emphasized by Greene and Blackford in 1926. We showed this to be true by determining first the icterus index of the untreated blood serum and then determining the index of the fraction of the serum treated with petroleum ether. The icterus index is always higher than the latter value when xanthemia is present.

Quantitative methods of determining the lipochrome pigments have been used before by other investigators. Reasonably accurate colorimetric methods were described by Willstatter and Stoll in 1913 and by van den Bergh and Muller in 1920. By the use of such methods values were determined after comparison of the extracted pigment in petroleum ether with a standard solution of potassium dichromate. Kohn in 1923 devised a spectrophotometric method for the quantitative determination of these pigments and Nienhaus in 1924 compared the blood serum colorimetrically against a standard solution of 2 per cent chromic acid. We decided however to determine only the relative amount of lipochrome pigments in the blood by colorimetric comparison of the yellow lipochrome pigment with the potassium dichromate standard (1:10,000 solution) which is used in the determination of the icterus index. We extracted the yellow lipochrome pigments from the blood serum by means of petroleum ether as practiced by van den Bergh and Muller. In short we determined the color index of the serum due to other pigments than bile pigments and expressed it quantitatively by a value which we designated the lipochrome index.

The directions which we followed in determining the lipochrome index were essentially as follows. To a measured amount of blood serum in a centrifuge tube add equal amounts of 95 per cent alcohol and petroleum ether shake well and centrifuge. Extract the pigments by the petroleum ether. Transfer the petroleum-ether layer to a graduated tube or small cylinder. Repeat the extraction of the serum as before until the petroleum ether layer is colorless removing this layer each time after centrifugation and adding it to the petroleum ether fraction previously removed. The petroleum ether fraction, containing the pigments is made up with more petroleum ether to a volume which gives a good depth of color for reading against the potassium dichromate standard employed for estimating the bile index as used by Meulengracht in 1921, Stetten in 1922, Bernheim in 1926 and others. Read in the colorimeter and calculate as follows:

$$\frac{\text{Standard set in mm}}{\text{Reading of unknown in mm}} \times \frac{\text{final dilution of unknown}}{\text{amount of serum used}} = \text{lipochrome index}$$

**Example.** Two c.c. of blood serum were used. Extraction was done and the volume was made up to 6 c.c. A reading of 10 was made against the potassium dichromate standard which was set at 15 mm. The calculation therefore is as follows:

$$\frac{15}{10} \times \frac{6}{2} = 4.5 \text{ or the lipochrome index}$$

Because of its simplicity this quantitative method permits a comparison of the icterus index with the lipochrome index, and by this means one can obtain a fair idea of the proportion of bile pigments and lipochrome pigments that produce color in the blood serum. In some of our cases the icterus index was omitted and only the lipochrome index was determined when the serum bilirubin content of the blood was within normal limits.

#### CLINICAL INVESTIGATION

The occurrence of xanthemia and xanthosis, as stated earlier in the paper, was first determined for a group of 100 diabetic patients, then for a group of 53 patients with other disease, and without diabetes, and finally for a group of 36 patients with xanthosis in whom the xanthosis occurred either independently or associated with some disease.

*Patients With Diabetes*—Of the 100 diabetic patients, 56 were males and 44, females. Their ages varied from four to seventy-four years, and they were distributed among the different age groups as shown in Table I. Sixty-nine per cent of the patients were between forty and seventy years of age, only 12 were in the first two decades of life.

1 Xanthemia. In 86 cases, 86 per cent, xanthemia occurred, it was absent in 14 cases. Practically all of the patients in the first four decades had xanthemia (Table I).

The results of investigations already referred to in the introduction of this paper have proved that the lipochrome pigments of the blood and skin in man originate from the ingestion of vegetables, fruits, butter, and eggs, in which these pigments are present. Since diabetic patients as a class partake of much of these foods, it was not surprising to find xanthemia in 86 of the 100 diabetic patients.

TABLE I

PRESENCE OF XANTHEMIA AMONG DIABETIC PATIENTS ARRANGED ACCORDING TO AGE GROUPS BY DECADES

AGE, YEARS	TOTAL CASES	XANTHEMIA		PER CENT POSITIVE
		POSITIVE	NEGATIVE	
0-9	2	2		100
10-19	10	10		100
20-29	11	10	1	91
30-39	5	5		100
40-49	20	17	3	85
50-59	25	20	5	80
60-69	24	20	4	83
70-79	3	2	1	66
Total	100	86	14	86

On their entrance to the diabetic service in the hospital, 27 of the 100 diabetic patients had been getting a normal diet, in 52, there had been a qualitative restriction of carbohydrates, and 21 had been receiving weighed diabetic diets. In the cases of the 21 patients who were getting weighed diets, rich in vegetables, tests for xanthemia were positive in 19 (90 per cent) and negative in two. In the 52 cases in which there was qualitative restriction, tests for xanthemia were positive in 45 (86 per cent), and negative in 7 (14 per cent), whereas of the 27 patients who received an ordinary, normal home diet, 22

(82 per cent) gave tests positive for xanthemia and five (18 per cent) gave negative tests

It is certain that the patients who received a weighed diet for diabetes were eating more vegetables and butter than those who received a qualitatively restricted diet, and those, in turn, were eating somewhat more vegetables and butter than those who were getting the normal diet. Xanthemia would be expected to be increasingly more prevalent among patients in proportion to the amount of the pigment containing foods they eat and this is shown to be true in this investigation. The surprising thing was that there should be two patients on weighed diets for diabetes who did not have xanthemia.

There were two patients who had been receiving a normal diet before coming to The Mayo Clinic where their diabetes was discovered and who did not have xanthemia on entrance to the hospital. In these two patients, however, xanthemia was discovered in tests after they had been receiving a diet for diabetes for twelve days and for three weeks respectively. The diet included from 600 to 800 gm of fruits and vegetables, one or two eggs and 30 to 60 gm of butter daily. There were two other patients who were known to have had diabetes, six months and nine months respectively, who had been getting practically normal diets; tests for xanthemia on these patients were negative on entrance to the hospital but they became positive on diabetic diets at the end of five and ten days respectively. Sixteen patients with diabetes did not have xanthemia, however, even though they had been on diets for diabetes, containing 600 to 800 gm of vegetables and fruit, one or two eggs, and 30 to 60 gm of butter, from one to three or more days at the time the lipochrome test was made. Some of these patients were re-examined later and still did not have xanthemia after being on a weighed diet for diabetes from six to thirty-two days.

Regarding the relation of the severity of the diabetes to xanthemia it was found that in the patients who had xanthemia cases varied from those which were mild, requiring only dietary management, to those requiring varying amounts of insulin. Some patients had had diabetes for as long as twenty years, others for only a brief period. Some were in a state of acidosis and coma on entrance to the hospital. A few had gangrene of the toes. The patients without xanthemia had had diabetes from just a few months to as long as twenty-two years. Two of these patients also had gangrene of one of the extremities. As a group these patients had mild or only moderately severe diabetes.

**2 Xanthosis.** In nine of the 100 diabetic patients who were examined xanthosis was observed. All of these 9 patients also were in the group of 86 with xanthemia. Xanthosis did not occur in any patient who did not have xanthemia. This is in accord with Salomon's observations. All of the 9 patients except one, in whom the xanthosis was faint, had moderately severe or severe diabetes and all required insulin except the one noted above. This observation is similar to that of Rabinowitch in 1928. Two of these patients had xanthoma diabeticorum as well and one of these had entered the hospital in coma.

None of these patients was obese, although it was in obese children that Klose, in 1919, thought xanthosis developed most readily. For this reason, Klose thought that xanthosis might be related to the fat metabolism of the body. With this idea in mind, blood-fat determinations were made on 18 patients with diabetes. The blood-fat determinations were made through the courtesy of Dr. Luden of The Mayo Clinic, according to her modification of the Bloor methods. In Table II are shown the values for cholesterol (Bloor II), fatty acids and total fats, as determined in these 18 cases, and also the normal values determined by the same methods.

Two patients (Cases 66 and 96) had definite lipemia and possessed high (Case 66) and extraordinarily high (Case 96) blood-fat values. These two patients also had xanthoma diabeticorum. Cholesterol values in the other 16 cases were quite within the normal range of variability, although in some there was a tendency for the cholesterol to become elevated. The fatty acids and total fats were generally high as might be expected in patients receiving diets relatively rich in fat. It should be noted, however, that while there were 17 cases of xanthemia among these 18 patients, xanthosis was present in only four. Two of these cases were in patients with lipemia, but in the other two cases (Cases 12 and 88) blood-fat values were similar to those of many of the cases without xanthosis. The data based on this small group of cases would, therefore, tend to indicate that the blood-fat values bear no definite relationship to xanthosis.

Table II also brings out the point that when the lipochrome-index is high as in Case 12, xanthosis is likely to occur, but it may also occur when the lipochrome-index is as low as 2.2, and it may be absent when the lipochrome-index is even higher, as in Case 94 in which it was 3.5. The lipochrome-index does not seem to bear any definite quantitative relationship to xanthosis and represents only a relative value for the amount of lipochrome pigments in the blood stream at the time the test is made.

TABLE II  
BLOOD FATS IN PATIENTS WITH DIABETES

CASE	XANTHOSIS	LIPOCHROME- INDEX	MG IN EACH 100 CC OF BLOOD		
			CHOLESTEROL, BLOOR II	FATTY ACIDS	TOTAL FAT
Normal values			115 to 180	100 to 130	200 to 300
9	-		128	222	350
10	-	1.7	128	244	372
11	-		108	158	266
12	+	5.0	145	320	465
26	-		108	222	330
66	+	2.2	307	555	862
67	-	2.8	157	317	474
83	-		65	180	245
84	-	2.2	157	202	359
85	-	3.2	175	202	377
86	-	0.5	157	317	474
87	-	2.8	193	370	563
88	+	2.7	89	139	228
90	-	1.5	177	268	445
93	-	2.6	108	158	266
94	-	3.5	118	171	289
96	+	4.8	954	8888	9842
97	-	1.7	134	180	314

3 Relationship of the bile and lipochrome pigments of the blood In the whole group of patients with diabetes the bilirubin content of the blood serum varied from less than 0.3 mg to 2.4 mg in each 100 c.c. of blood The serum bilirubin was within normal limits (0.2 to 2.0 mg in each 100 c.c.) in all cases with the exception of one It averaged 0.76 mg in each 100 c.c. for the entire group The icterus index varied from 3 to 135, in nine cases it was 10 or more and the average was 6.9 for the entire group This is slightly above the upper limit of normal which is 5

In 13 cases the serum bilirubin the icterus index and the lipochrome index were determined at the same time In all of the cases, the serum bilirubin was within normal limits the highest value was 1.3 mg, the icterus index varied from 3 to 135, and the average was 7.3 for the group of 13 cases The lipochrome index varied from 0.5 to 5 and averaged 3.5 for the whole group

The relationship of the serum bilirubin icterus index and lipochrome index to each other is shown in Table III

TABLE III

RELATIONSHIPS OF SERUM BILIRUBIN ICTERUS INDEX AND LIPOCHROME INDEX IN PATIENTS WITH DIABETES

CASE	SERUM BILIRUBIN MG IN EACH 100 C.C.	ICTERUS INDEX	LIPOCHROME INDEX	LIPOCHROME INDEX CALCULATED IN PER CENT OF ICTERUS INDEX
12	1.2	0.0	5.0	55
66	1.3	7.7	2.2	28
67	0.7	5.9	2.8	47
84	0.3	5.0	2.2	45
85	0.3	7.5	3.2	43
86	0.0	4.8	0.5	10
87	0.7	8.8	2.8	32
88	0.5	6.3	2.7	43
90	0.3	3.0	1.5	50
93	0.3	5.0	2.6	52
94	0.3	6.8	3.5	52
96		13.5	4.8	36
99	1.3	9.6	3.0	31
100	1.1	9.8	1.6	16

The significant point in Table III is that the lipochrome index was from 10 to 55 per cent of the icterus index value It averaged 38 per cent for all the cases It is important, therefore when evaluating the icterus index to realize that probably one third to one half of the color of the serum is due to the presence of lipochrome pigments

To summarize (1) 100 patients with diabetes were examined qualitatively for the presence of lipochrome pigments in the blood and skin of which 86 had xanthemia and 9, xanthosis (2) the lipochrome index averaged about 38 per cent of the icterus index which indicates that much of the color of the blood serum can be attributed to the lipochrome pigments of exogenous origin, (3) the presence in the diet of foods containing these pigments was undoubtedly a

The van den Bergh technic for determining serum bilirubin quantitatively admittedly is inaccurate when the values are lower than 1 mg for each 100 c.c. of blood This is owing to the difficulty of accurate matching of colors in the colorimeter The figures for serum bilirubin in Table II probably possess an inaccuracy of not greater than + or - 10 per cent The error involved does not affect the conclusions



large factor in the production of xanthemia, but other factors must be involved too, since some patients on diets rich in vegetables, fruits, butter, and eggs failed to have xanthemia, this finding may be related to the individual variation in the factors which regulate the absorption, circulation, deposition in the skin, oxidation and excretion from the human body of the lipochrome pigments, (4) xanthosis did not occur in the absence of xanthemia, but xanthemia often occurred without xanthosis, (5) xanthosis occurred more often in the severe cases of diabetes, (6) xanthemia did not seem to be related to the amount of lipoids in the blood except perhaps in lipemic patients, who also had severe diabetes, and (7) there was no quantitative relationship between the height of lipochrome-index and the presence or absence of xanthosis

*Patients Without Diabetes*—A series of 53 patients without diabetes but with other illnesses was examined for evidence of xanthemia and xanthosis. There were 13 patients with disease of the liver or biliary tract, 22 with renal disease, 3 with diagnoses of chronic nervous exhaustion, 2 with renal glycosuria, 2 with Addison's disease, 2 with orthostatic albuminuria, 3 with arthritis, one with exophthalmic goiter, one with lymphosarcoma, one with carcinoma of the stomach, one with static edema, one with hemolytic jaundice, and one with chronic appendicitis. Thirteen were females and 40 were males. Their ages varied from sixteen to sixty-nine years. Forty-two were from thirty to sixty years of age. Of this group, 47 (88 per cent) had xanthemia, and xanthosis was present in three (5.5 per cent).

Of the 13 patients with disease of the liver or biliary tract, all were in the hospital, where a study was being made to determine the etiology of their condition. Eleven of them were jaundiced. The cause of the jaundice was either stone in the common duct, stricture of the common duct, hepatitis (catarrhal or epidemic), cirrhosis of the liver, or metastasis to the liver. The serum-bilirubin content of the blood varied from 1.1 to 25 mg. for each 100 cc. Only two patients had a normal serum-bilirubin, and these were not jaundiced at the time the test for lipochrome pigments was made, although the van den Bergh reaction was direct in both as it was in the 11 jaundiced patients. Xanthemia was present in 9 cases (69 per cent) and absent in 4 (31 per cent). It was not present in two cases of obstruction of the common duct, in one case of catarrhal jaundice, or in one case of pelvic and abdominal carcinomatosis with metastasis to the liver. Xanthosis was not present in the two patients who were not jaundiced, and it could not be determined because of the icterus in the jaundiced patients. All the patients in this group had been receiving normal diets before coming to the hospital, with the exception of one who had eliminated fats from the diet.

The group of 22 with renal disease was made up of patients with chronic nephritis, or with generalized or renal arteriosclerosis often associated with hypertension and myocardial degeneration. All of these patients had xanthemia, and two, xanthosis. One of the two patients with xanthosis had chronic glomerulonephritis, and the other, malignant hypertension with renal insufficiency. Sixteen of these patients had been receiving normal diets be-

fore coming to the hospital and only six had been getting diets for nephritis, low in proteins and probably high in vegetables and fruits. The incidence of xanthosis, 9 per cent (two cases) is identical with that among the diabetic patients examined. It occurred in one patient who had been getting a normal diet, which contained only an average amount of vegetables, and in another patient who had been eating much vegetable soup for four months.

There is still a group of miscellaneous cases in which, as in the previous two groups, diabetes was not concerned. In sixteen cases (89 per cent) there was xanthemia and in one case (11 per cent) xanthosis. There were two patients with renal glycosuria, both of whom had been on weighed diets for diabetes for some time. One had a serum bilirubin of 0.7 mg. in each 100 cc., an icterus index of 97, without jaundice and a hypochrome index of 43 without xanthosis. This case is especially interesting because in some diabetic cases with a lower hypochrome index there was xanthosis. In two cases each of Addison's disease, orthostatic albuminuria and chronic arthritis, and in three of chronic nervous exhaustion xanthemia without xanthosis was present. Likewise, xanthemia was found in one case each of exophthalmic goiter, carcinoma of the stomach, static edema, hemolytic jaundice and chronic appendicitis. The patient with chronic appendicitis also had xanthosis, he had been on a high vegetable diet and the yellow color in the skin had been present for two weeks. In one case of chronic arthritis and one of lymphosarcoma tests for xanthemia were negative. All of these patients with the exception of the one with chronic appendicitis had been getting normal diets before coming to the clinic.

In summarizing the results of the study of this group, composed of 53 cases of various diseases other than diabetes we found that (1) xanthemia was present in 47 cases (88 per cent); this figure is almost identical with that obtained in cases of diabetes (86 per cent), a normal diet had been given in 31 of these. (2) xanthosis occurred in only three cases (5.5 per cent), little more than half as frequently as in the cases of diabetes, (3) xanthemia occurred in the jaundiced patients about as frequently as in those who were not jaundiced, (4) patients with renal insufficiency due to nephritis, or associated with arteriosclerosis and hypertension all had xanthemia even though the majority apparently were receiving normal diets, (5) two of the 22 patients with renal disease (9 per cent) had xanthosis and this incidence was similar to that found among diabetic patients, but the number examined was smaller; one of these patients with xanthosis had been on a normal diet but the other one had eaten an excessive amount of vegetables for four months. (6) one patient with renal glycosuria who had been getting a weighed diet for diabetes for three months did not have xanthosis, and the hypochrome index was as high as 44 (serum bilirubin 0.7 mg.) this was surprising since some patients with diabetes and a lower hypochrome index had xanthosis.

*Thirty-six Patients With Xanthosis*—We reviewed 36 cases that had been diagnosed as xanthosis at The Mayo Clinic. There were 27 males and 9 females in the group. Their ages ranged from twelve to seventy years. Four patients were between ten and nineteen years of age, 4 between twenty and twenty-nine years, 9 between thirty and thirty-nine years, 12 between forty

and forty-nine years, 4, between fifty and fifty-nine years, 2, between sixty and sixty-nine years, and one was seventy years of age

The chief complaints varied considerably in ten cases it was diabetes, in twelve, stomach trouble, in five, weakness, in two, jaundice, and in one each it was one of the following nervousness, vertigo hypertension, asthma, goiter with throbbing in the neck, pain in the left lower quadrant, and constipation. The degree of xanthosis in these patients varied from definite yellowing of the palms, soles and nasolabial folds, to a more generalized yellow pigmentation of the skin.

It was thought advisable to learn the influence that this pigmentation had in determining the direction of the clinician's investigation. Naturally, the first supposition was that the color represented jaundice, and with this, the commonest possibility of cholecystitis. In this group of 36 patients, 10 had diabetes, and cholecystitis with jaundice was not considered a possibility in any of these, but 12 other patients complained of stomach trouble, biliousness or upper abdominal distress. Two of the latter group were sent to the clinic by their local physician with the diagnosis of suspected cholecystitis with jaundice. Six of these cases actually were investigated for cholecystitis at the clinic, but in the other 6, the diagnosis of xanthosis was made at once. In none of the 6 cases investigated for gall bladder disease was an ultimate diagnosis of cholecystitis made. It was apparent from the study of these 12 cases that when a patient with xanthosis complains of stomach trouble or upper abdominal disturbance, jaundice with cholecystitis often will be suspected by the clinician unfamiliar with the picture of xanthosis and probably will be investigated from that point of view.

The question of cholecystitis was raised, however, in three other patients who had not complained of upper abdominal distress. One patient complained of nervousness and another complained of pain in the left lower quadrant. Both of these had been on high vegetable diets for several months, eating many carrots each day. The ultimate primary diagnosis was hypochondriasis in the former case and only xanthosis in the latter case. The third patient complained of constipation. This patient was the only one in whom, ultimately, cholecystitis was diagnosed. The full and final diagnosis was neurosis, xanthosis and cholecystitis. The diagnosis of cholecystitis was based on the elicitation of slight tenderness in the right upper quadrant and an invisible gall bladder with the Graham-Cole technique. There was no history of gallstone colic. The serum-bilirubin had not been investigated and the sclerotics were clear. The fact that the patient had been eating carrots daily for four months for her constipation readily explained the color of her skin, which was deepest in the palms and soles. There is only a question here, suggested by the roentgenogram, whether this patient actually had cholecystitis, clinically there was no evidence of it, except the slight tenderness in the upper quadrant, but even this may be partly discounted in view of the constipation and also the neurosis noted in the diagnosis.

Xanthosis was the sole diagnosis in four cases. The diagnosis of xanthosis was accompanied, however, by a final diagnosis of diabetes in ten, chronic nervous exhaustion in two, adenomatous goiter in one case, chronic appendi-

itis in two cases with operation in both, chronic glomerulonephritis in two, achylia gastrica in two, "functional stomach" in two, latent syphilis in one case, malignant hypertension in one, asthma in one, duodenal ulcer in one, cardiac neurosis in one, uterine myomas in two cases, chronic infectious arthritis in one case, neuritis and cholecystitis in one case and menopausal neurosis in one case

The yellow discoloration of the skin also led the local physicians, in two cases, to suspect Addison's disease. One of these cases finally was diagnosed as exhaustion with hypotension and the other only as xanthosis. Two cases were investigated in the clinic for pernicious anemia but were later diagnosed as migraine and chronic glomerulonephritis. In another case, the question of chronic glomerulonephritis was raised and finally was diagnosed chronic glomerulonephritis and xanthosis.

The fact that 10 of the 36 patients had diabetes shows that this condition was more prevalent among such patients than among other persons who were ill or in good health. Even this number of patients with diabetes is smaller than the actual number since the condition is so comparatively common in such patients that it is not always indexed.

The serum bilirubin was investigated in 19 of the 36 cases. The highest value was 2.8 mg. and the lowest 0.1 mg. with an average of 0.7 mg. The icterus index was determined in 14 cases and varied from 30 to 150, with an average of 100. The high average (normal limits 1 to 5 inclusive) can be attributed to the extra color contributed to the blood serum by the presence of the lipochrome pigments in the blood stream, since the serum bilirubin values were practically within normal limits. These data in the cases investigated, along with the clinical signs and symptoms, excluded the diagnosis of cholecystitis with jaundice and afforded a valuable aid in making the diagnosis of xanthosis.

It is important to elicit as much information as possible concerning the diet of such patients, for the reason that the majority of those with xanthosis had been on a high vegetable diet for some time. Of the 36 patients with xanthosis 31 had been on a high vegetable diet for various reasons. One patient had been on a diet for nephritis, one patient who was ambulatory had been getting a diet for peptic ulcer and one had been receiving a general diet. Information was not collected regarding the diet of two patients.

Eleven of the patients with xanthosis had been eating excessively of carrots, four of eggs and three of spinach. Knowledge of the diet and the characteristic distribution of the pigment in the palms, soles and nasolabial folds, but not in the sclerotics, are sufficient to identify the color as that of xanthosis and to rule out jaundice as a possibility. Should the sclerotics be icteric the possibility of both xanthosis and jaundice should be considered.

A summary of the study of the 36 cases of xanthosis\* showed it to be

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Since the completion of this study two other cases of xanthosis have been observed. In one the diagnosis established was chronic nephritis and in the other nervous exhaustion. Both of these patients had been receiving diets rich in lipochrome pigment. The patient with nervous exhaustion had eaten an average of 150 gm. of carrots daily for ten months and had had xanthosis for nine months. The other patient had been on a low protein high vegetable diet for three years and had had xanthosis during the last eighteen months. These cases are remarkable for their high lipochrome-index. It was 1 in the patient with nervous exhaustion and 8 in the patient with nephrosis. The serum bilirubin values were normal. The xanthosis of the patient with nervous exhaustion had been noted to vary from time to time in intensity.

icates a poor prognosis. We cannot quite agree with this because (1) the more severe the diabetes the more rigid as a rule is the diet and such diets are high in lipochrome content, (2) xanthosis did not influence the prognosis in 26 patients without diabetes, and (3) experimentally induced xanthosis is harmless.

#### SUMMARY AND CONCLUSIONS

1 The relative amount of lipochrome pigments contained in the blood plasma, a lipochrome-index, can be readily determined by the simple quantitative method described. Direct comparison with the icterus-index is possible and in many cases we have found the lipochrome pigments contributing from 10 to 50 per cent of the color of the blood plasma.

2 One hundred patients with diabetes and 53 patients suffering from other diseases were chosen at random and investigated for xanthemia and xanthosis. Xanthemia was found in 86 (86 per cent) of the patients with diabetes, in 22 (100 per cent) of patients with renal disease, in 13 (69 per cent) of patients with hepatic disease, and in 16 (89 per cent) of patients with other miscellaneous diseases. Xanthosis was present in 9 (9 per cent) of the patients with diabetes, in 2 (9 per cent) of the patients with renal disease and in one (3 per cent) of the patients with other diseases.

3 Among 36 patients whose condition was diagnosed as xanthosis were 10 with diabetes and 26 who had other diseases or, with the exception of the xanthosis, were normal. The majority of these patients had eaten excessively of foods rich in the lipochrome pigments, namely, vegetables, fruits, butter, and eggs.

4 The evidence does not warrant the conclusion that the presence of xanthosis as a complication of diabetes affects the course of the disease unfavorably. It is probable, however, that the ability of the individual to oxidize and excrete the lipochrome pigments varies and that there are some factors in some patients with diabetes that interfere with these processes, such factors may be acidosis and dehydration of the skin.

5 The yellow color of the skin in xanthosis may lead to confusion in diagnosis through its being mistaken for icterus. The distribution of the pigment in xanthosis is characteristic. The coloring is most intense in the palms, soles, and nasolabial folds, whereas the sclerotics usually are clear. A history of the patient's diet may be helpful in explaining the pigment, and determination of the lipochrome index, icterus-index and serum-bilirubin permits of conclusive diagnosis. When yellow pigmentation of the skin is present and the serum-bilirubin is low and the icterus-index high, the condition must be xanthosis, if the serum-bilirubin is high and the lipochrome-index low, the abnormal coloration is due to jaundice, if both the serum-bilirubin and lipochrome-index are elevated, xanthosis is present, but is obscured by jaundice.\*

\*We regret that the paper by Stannus which appeared in the *International Clinics* for March 1929 reached us too late for review. Any reference now will necessarily be inadequate because the paper is an excellent review of xanthosis. Stannus like ourselves concludes that xanthosis results from a failure to oxidize the carotinoid pigments in the body although as we have stated this may be only one factor in the production of this condition. We do not favor the adoption of the term hyperlipochromia as Stannus suggests to supplant the term xanthosis. Our reason has been given in our paper.

## REFERENCES

- 1 van den Bergh A A H, and Muller P Das lipochrome Pigment in Blutserum und Organen, Xanthosis, Hyperlipochromämie Biochem Ztschr 108 279 303, 1920
- 2 van den Bergh A A H and Snapper J Die Farbstoffe des Blutserums Deutsch Arch f klin Med 110 540 561 1913
- 3 Bernheim, A R The Significance of Variations in Bilirubinemia Arch Path & Lab Med 1 747, 58 1926
- 4 Connor, C L Studies on Lipochromes I The Reaction of Animals to the Presence of Carotin Am J Path 4 22, 23, 1928
- 5 Greene C H, and Blackford L M Carotinemia Med Clin N Amer 10 733 744, 1926
- 6 Hashimoto, Hirotsuki Carotinoid Pigmentation of the Skin Resulting From a Vegetarian diet J A M A 78 1111 1113 1922
- 7 Hayem G Coloration spéciale des téguments chez certains dyspeptiques ictere sans pigments biliaires ni urobiline dans les urines Ann de dermat et de syph 28 793 794 189,
- 8 Head G D and Johnson R A Carotinemia Report of a Case in an Adult Arch Int Med 28 268 273 1921
- 9 Hess A F and Myers, V C Carotinemia A New Clinical Picture, J A M A 73 1743 1745, 1919
- 10 Klose Erich Hautverfärbung bei Säuglingen und Kleinkindern infolge der Nahrung München med Wehnschr 1 419 1919
- 11 Kohn Bertha Karotingerbsucht bei Kindern Ztschr f d ges exper Med 36 447 456 1923
- 12 Meulengracht E Ein Bilirubinometer behufs klinischer Bestimmung der Bilirubinmenge im Blute Deutsch Arch f klin Med 137 38 46 1921
- 13 Miura K Carotin als Ursache von Aurantiosis Cutis (Ba litz) Festschr gewidmet Keizo Dohi 2 203 January 1917
- 14 Miyake Isam Ueber Aurantiosis cutis Brault Arch f Dermat u Syph 147 184 190 1924
- 15 Maro E Karottensuppe bei Ernährungsstörungen der Säuglinge München med Wehnschr 2 1637 1640 1908
- 16 Niehaus F W Case of Carotinemia J A M A 82 547 1924
- 17 von Naarden Carl Die Zuckerkrankheit ed 4, 1907 Berlin A Hirschwald p 149
- 18 Palmer, L S Carotinoids and Related Pigments the Chromolipids 1922 New York Chemical Catalog Co p 316
- 19 Palmer, L S and Kempster H L The Physiological Relation Between Fecundity and the Natural Yellow Pigmentation of Certain Breeds of Fowl J Biol Chem 39 313 330, 1919
- 20 Rabinawitch I M Carotinemia and Diabetes, Canada Med Assn J 18 527 530 1928
- 21 Saloman H Ueber Pseudokterus nach Mahlrubengenuß München med Wehnschr 1 564, 1919
- 22 Salaman, H Ueber Xanthose der Haut namentlich bei gesunden Leuten und über Xanthamie, Wien klin Wehnschr 32 495 49, 1919
- 23 Stannus H S Hyperlipochromin (Carotinemia Xanthosis cutis) Internat Clinics Series 39 1 146 163, 1929
- 24 Stetten De Witt The Surgical Value of the Estimation of the Bile Pigmentation (Icterus Index) of the Blood Serum Ann Surg 76 191 200 1922
- 25 Stoner, W C Carotinemia Am J Med Sc 175 31 36 1925
- 26 Wells H G and Hedenburgh O F The Toxicity of Carotin J Biol Chem 27 213 216 1916
- 27 Willstatter Richard and Stoll Arthur Untersuchungen über Chlorophyll 1913 Berlin Springer, p 494

# THE RÔLE OF STREPTOCOCCI IN THE RHEUMATIC DISEASES\*

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IN ADDRESSING the Bath Conference on Rheumatic Diseases in May, 1928, Poynton<sup>1</sup> expressed the hope that "there will not be a splitting up of the study into heart, joint, and chorea units, for the constitutional disease and not the isolated lesion is still the main problem." This statement from so illustrious a pioneer in the study of rheumatic fever holds particular significance. It expresses that broad conception of the disease which is so essential if progress is to be made in elucidating its cause, or in combating it effectively.

Rheumatic fever should not be considered as an acute arthritis, as a disease of the heart, nor yet as a disease of any particular system or group of organs, but as a general disease producing characteristic pathologic lesions of widespread distribution. From the standpoint of the pathologist, it may be regarded as a disease producing lesions in endothelial structures wherever these are found in the organism. In a more restricted sense, it may be considered as a disease producing lesions in and adjacent to the blood vessels and consequently its lesions occur in any tissue penetrated by even the smallest capillary.

From the clinical standpoint we have been too prone to think of the acute arthritis as the disease, rather than as an outstanding symptom of the disease. One may yet hear the cardiac manifestations spoken of as "complications" of rheumatic fever. This in a measure is excusable since from both the clinical and pathologic points of view, lesions of two types are recognized. These are designated the "exudative" lesions, best exemplified by the acute arthritis, and the "proliferative" lesions typified in their purest form in the subacute, progressive cardiac lesions. There is no doubt but that the former are promptly affected in a favorable manner by the administration of salicylates. Close students of the disease are in accord in believing that the latter type of lesion is not favorably affected by salicylates. Here then we have one of the enigmas of the disease, upon the solution of which hinges certain very puzzling questions of its etiology and pathogenesis. This situation at once suggests that the discovery of the agent will not clear up the subject, and that great difficulties would yet be encountered in determining the *modus operandi* by which the agent produces two types of lesions which differ so essentially in their characteristics as well as in their responses to a given form of therapy.

There is a third type of lesion which is likely to be overlooked. It is the "destructive" lesion. It is studied best in the heart muscle of patients who have died early in an attack of rheumatic fever, attended by an overwhelm-

\*From the Laboratories of the Philadelphia General Hospital.  
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ing clinical toxemia This lesion amounts to actual sterile necrosis of the muscle fibers, occurring in areas of a considerable extent

The belief that rheumatic fever is an infectious disease has much in its support The clinical manifestations of the acute attack suggest this The seasonal occurrence of the disease is well known The cyclic appearance of multiple cases in families has been emphasized by St Lawrence<sup>2</sup> and by Faulkner and White<sup>3</sup> The association of the onset of the disease with attacks of acute tonsillitis, together with the many bacteriologic studies associating streptococci with the etiology of tonsillitis, has supported the connection between streptococci and rheumatic fever as first emphasized by Poynton and Payne<sup>4</sup> Atwater<sup>5</sup> recently has shown in epidemiologic analyses that the curves for the incidence of streptococcus infections and those for the prevalence of rheumatic fever run parallel in studies of the seasonal and geographic occurrences of these diseases

Cultures of the blood in the acute attack of rheumatic fever have aided little, because it has been the common experience that these are in a large part sterile Streptococci have been isolated perhaps more frequently than any other bacteria, but there has been no definite agreement in the types of streptococci as between the different workers or as between the strains isolated by the individual worker Yet various as have been the strains isolated, experimental animals inoculated with them have developed acute arthritis The different members of the streptococcus group, therefore, appear to have this feature in common that they tend to produce arthritis in rabbits It must be emphasized, however, that this experimental streptococcus arthritis has been for the most part a suppurative arthritis and quite different from the acute arthritis of rheumatic fever

The exact reproduction of the microscopic lesion of the myocardium in rheumatic fever (nodule of Aschoff) has not been accomplished beyond dispute Coombs,<sup>6</sup> de Vecchi,<sup>7</sup> Jackson<sup>8</sup> and Cecil<sup>9</sup> have produced myocardial lesions in rabbits closely resembling the nodules of Aschoff Greatest attention has been given to the green producing streptococci in all of this work, but we do find some who have dealt with the hemolytic and with nonhemolytic streptococci Rosenow<sup>10</sup> in 1914 reported the isolation of three strains of streptococci which produced no effect upon the blood in the media upon which they were grown He showed that these streptococci were capable of producing experimental lesions in animals

In January, 1927<sup>11</sup> I reported the isolation of an indifferent streptococcus in cases of rheumatic fever It was obtained in the first instance from a blood culture, and later with great regularity from cultures of the pharynx in cases of rheumatic fever or of chorea A large number of these strains have been collected A recent report on the biologic and serologic relationships of 107 strains by Kreidler,<sup>1</sup> confirms my earlier observations on the compact nature of this group They form the first compact serologic group of streptococci which has been associated with rheumatic fever, a fact alone which should stimulate considerable interest in this microorganism The organism was named *Streptococcus cardioarthritis*

Further studies of this streptococcus,<sup>12</sup> demonstrated its intimate associa-



tion with the disease, rheumatic fever, its widespread geographic distribution, its ability to produce the tissue reactions and the lesions in rabbits and horses, which one is unable to distinguish from those of rheumatic fever, as has recently been emphasized by the work of Belk, Jodzis and Fendrick.<sup>14</sup> Its immune bodies (agglutinins and opsonins) are readily demonstrated in the sera of patients with rheumatic fever. Vaccines and soluble products of cultures of this streptococcus have been shown to precipitate acute exacerbations of rheumatic fever in individuals with low grade quiescent forms of the disease. Such exacerbations have been attended not only by acute arthritis, but by pericarditis, and the development of crops of subcutaneous nodules. The antiserum prepared from a single strain of this streptococcus has been employed over a period of two years in the treatment of patients, with results which have been more and more encouraging. The antiserum at first employed in relatively large volumes in an unconcentrated form, has later been concentrated by the globulin precipitation method until the volume of adequate dosage for the individual case has been reduced to 10 c.c. or less.

Closely following the original description of *Streptococcus cardioarthritidis*, Birkhaug<sup>15</sup> reported the association of nonhemolytic, nonmethemoglobin forming streptococci with rheumatic fever. From his description as well as from the reactions in the fermentation of carbohydrates, it is evident that his series contained strains of *Streptococcus cardioarthritidis*. This is further strengthened by his later work<sup>16</sup> in showing the effect of the antiserum of his strain (R F 24) in neutralizing the "primary" skin reactions of animals sensitized with *Streptococcus cardioarthritidis*.

Hitchcock<sup>16</sup> has also collected a number of strains of the anhemolytic streptococci, and has recorded studies which show that serologically specific species, other than *Streptococcus cardioarthritidis*, occur in this group. Unpublished studies by Seiger in our laboratory, have enabled him to identify serologically specific species of indifferent streptococci in addition to *Streptococcus cardioarthritidis*. The studies of these workers show that *Streptococcus cardioarthritidis* is only one of a number of distinct species of streptococci which neither hemolyze blood, nor produce methemoglobin in artificial culture. They also emphasize the importance of the serologic tests in the identification of *Streptococcus cardioarthritidis*.

Lazarus Barlow<sup>17</sup> at the International Conference on Rheumatic Diseases at Bath, England, in May, 1928, presented a preliminary report on the neutralizing effects of antiserum upon culture filtrates of strains of indifferent streptococci isolated by himself, by Birkhaug and myself. From a study of the toxic effects of these filtrates on rabbits, his evidence indicated that a given antiserum, neutralized the filtrates of cultures of each of these strains with equal facility. This is an observation which if substantiated in further studies is important in fixing the identity of these strains isolated in widely different localities.

The studies presented in the recently issued Volume<sup>18</sup> of the Pickett-Thomson Research Laboratories stressed the identity of *Streptococcus cardioarthritidis*, and the strains supplied by Birkhaug and by L. Barlow, and states

that they are all closely related to Warren Crowe's Group A (Variety 3 or 4)<sup>10</sup> of anhemolytic streptococci

H Lowenberg,<sup>9</sup> of Berlin, has recently described independently a serologically specific streptococcus isolated from the pharyngeal cultures of rheumatic patients and of normal individuals. He designated this, *pharyngokokkus*. His report which includes the description of the cultural characters as well as a record of tests by agglutination and absorption of agglutinins of twenty three strains of this streptococcus was prepared before the description of *Streptococcus cardioarthritidis* came to his attention. Upon the receipt of my strains he found that they conform both in cultural characters and in serologic reactions so that he concludes that there is no doubt of the identity of *pharyngokokkus* and *Streptococcus cardioarthritidis*.<sup>21</sup>

Since the work of Herry<sup>22</sup> in 1914 and particularly that of Faber<sup>3</sup> in 1915, there has been a growing tendency to explain the manifestations of rheumatic fever on the basis of bacterial allergy. The excellent and extensive work of Swift<sup>24</sup> and his coworkers has been important in developing facts in regard to this theory. In a late report Swift<sup>2</sup> definitely declared himself in favor of the allergic theory. Zinsser and Yu<sup>6</sup> in a recent report also express this conception of the disease.

This conception differs essentially from those which attribute the disease to a bacteremia or a toxemia in that, while it associates the etiology of rheumatic fever definitely with streptococci, it declares in favor of no particular species or type strain of these microorganisms, since the allergic reactions may be excited by streptococci which differ widely both in biologic characters and in serologic relationships. That is to say that animals sensitized to one variety of streptococci react not only to the homologous antigen, but to antigens derived from heterologous strains.

Zinsser and Yu<sup>6</sup> do not attempt an explanation of the mechanism of this bacterial allergy further than to say that evidence seems to us to point toward the conclusion that the allergic state is a part of the immunologic mechanism, and in its first stages, before antibodies have been accumulated to any extent in the circulation, the phenomenon is mainly cellular expressing itself in an extraordinary irritability of the cells to contact with the antigen. That with some bacteria such as streptococci they may later develop into a true immunity, or anergy, in other words a state in which contact with antigen does not produce reactions."

Dochez and Stevens<sup>7</sup> have described in animals which had received injections of streptococcus of erysipelas, the existence of two types or phases of hypersensitiveness: one to the toxin which can be neutralized by antiserum in vitro, the other, to some other bacterial product which they were unable to neutralize. The first sensitizing antigen was destroyed by heat and was therefore referred to as a toxin, while the second was thermostable. Birlhaug<sup>8</sup> found that animals sensitized with his nonmethemoglobin forming streptococcus (Strain R F 24) showed likewise two phases of sensitivity to culture filtrates. The first appeared specific in that it was elicited only by filtrates of the homologous strain, and by those of *Streptococcus cardioarthritidis*. Homologous and crossed neutralization of the filtrates of these two strains was ef-

fects by their antiserum, while heating of the filtrates at 98° C for four hours completely eliminated the skin reaction in the earliest stages of this sensitization phase. During the second phase of sensitization neither antiserum nor heat had any effect in eliminating the skin reactions. During this stage also the animals reacted with positive skin tests to products of heterologous bacteria, *Streptococcus viridans*, *Streptococcus hemolyticus* of scarlet fever and erysipelas and pneumococci.

These observations suggest that the products of streptococci are complex substances, containing a multiplicity of protein substances each of which may act as antigen to produce its particular type of hypersensitiveness. In this particular they appear similar to horse serum or to egg albumen concerning which Von Dungern<sup>29</sup> has brought forth evidence to show that they contain a multiplicity of protein substances, each of which acts as antigen for the production of its own specific precipitins. The precipitins do not appear in the blood serum of the immunized animal in the same relative proportions in which the antigens occur in the horse serum, or in the egg albumen. Upon this observation then might hinge the explanation of the late appearance of the secondary phase of hypersensitiveness in animals treated with the complex antigens of streptococci.

Dochez and Stevens,<sup>27</sup> and Bukhaug's<sup>28</sup> observations that the late secondary phase of this hypersensitiveness is nonspecific, i. e., it appears as a hypersensitiveness to quite diverse serologic strains of streptococci, which also produce quite different toxic products, and appears also to pneumococcus, suggests that the bacterial bodies of such diverse microorganisms have in common some highly organized protein, which acts as a "protein specific" antigen rather than as a "species specific" antigen. We are familiar with such antigens from the work done with lens substance of the vertebrates, the immunologic specificity of which is not limited to a particular species, but occurs through a wide range of animal species as an "organ specific" antigen.

Swift's work in sensitizing animals with the nucleoproteins of streptococci, in which he finds that the antigenic properties of these cross the bars of immunologic type specificity in the streptococcus group and even invades the pneumococcus group, might be taken as evidence that in the nucleoproteins of bacteria (at least the gram positive cocci) we have such a "protein specific" antigen. The failure to find evidence of joint lesions, and of cardiac lesions in animals exhibiting extreme grades of this hypersensitiveness to nucleoproteins, as well as the failure to elicit focal symptoms referable to the joints and heart in patients with rheumatic disease, raises a serious doubt as to whether this type of hypersensitiveness has a significant part in the causation of the disease. The exhibition of the cross sensitization of this type between the streptococcus and the pneumococcus group, constitutes an argument for those supporting the view of nonspecific bacterial allergy as a dominant factor in the pathogenesis of rheumatic fever, that the disease might as readily be caused by pneumococci as by streptococci. Such reasoning, however, would do much to break down our whole conception of the specificity of bacterial disease which is too well grounded to be shattered by an imperfectly understood laboratory observation.

THE AUTHOR'S HYPOTHESIS REGARDING THE FACTORS CONCERNED IN  
THE ETIOLOGY OF RHEUMATIC FEVER

The present state of our knowledge concerning the etiology and pathogenesis of rheumatic fever is too fragmentary to formulate any definite underlying principles except on the basis of hypothesis. There seems to be rather definite evidence that streptococci are the bacteria concerned in its causation. The evidence which my studies have brought forward suggests that a specific streptococcus is primarily responsible. The work of Swift<sup>24</sup> and his coworkers of Zinsser and Yu<sup>6</sup> of Irving-Jones<sup>30</sup> and others favors the view that a specific streptococcus is not necessarily concerned.

I am given the credit of favoring the type specific strain of streptococcus as the cause of rheumatic fever acting through the specific toxic product alone. This but imperfectly presents my idea of the disease. It has been largely through the writings of others that my accredited position has been assigned to me. Swift is not convinced of a specific toxic element, and declares in favor of the nonspecific allergy to the various varieties of streptococci.

The studies of patients with rheumatic fever during the past two years have forced upon me the conclusion that there is an element of specificity to the *Streptococcus cardioarthritidis* in rheumatic fever. This element of specificity seems to be concerned with the 'destructive' and 'proliferative' lesions more so than the 'exudative' ones. The proliferative lesion is the most important lesion in rheumatic fever, since it is the lesion most concerned in producing cardiac damage and since we have in salicylates a means of controlling the exudative lesion. The proliferative lesion furthermore is the lesion upon which the clinical and pathologic definition of the disease so largely depends.

It is desired to state clearly my present working hypothesis in regard to rheumatic fever which is that the 'destructive' and 'proliferative' lesions are the result of a specific toxic product (endotoxin) derived from a particular group of streptococci. The destructive lesion is brought about by high concentrations of this toxin, the proliferative lesion, because of the stimulating effects of weaker concentrations. The exudative lesion appears when the patient begins to develop immunity to the specific toxic factor and is brought about by the establishment of a condition of hypersensitiveness which is a manifestation of the Arthus Phenomenon<sup>31</sup>.

From the work cited above it appears very likely that there are multiple factors concerned in this hypersensitiveness some of which may be bacterial species specific others specific only to certain highly specialized bacterial proteins. Because of the multiple antigens contained in streptococci namely the specific endotoxin and the protein factors one must presuppose the existence of separate and distinct antigen antibody complexes. Of these the antigen antibody complexes of the protein factors appear to be the most powerful irritating substances and would naturally be regarded as the dominant allergic factor, admitting the presence of other less potent allergic factors e.g. the toxin antitoxin complex.

This conception in no way disturbs the specificity of the destructive and proliferative lesions of rheumatic fever but at the same time, it indicates

that the exudate lesions may be accounted for on the basis of bacterial "protein" specificity and not on the basis of bacterial group, or type specificity. It furthermore relegates the exudative lesions to a secondary, purely concomitant rôle in the disease and permits the presumption that any microorganism supplying the "protein specific" antigen to which the patient has developed a hypersensitivity might play a part in determining the severity of the exudative lesions.

Fig 1 presents a graphic representation of the interrelation of toxic, antitoxic, and allergic factors as applied in our hypothesis to the rheumatic diseases. It represents the allergic factor as increasing at the same rate as the antitoxic factor. The antitoxic factor is represented on a different base line from the toxic and allergic factors, because by this means its effect in counteracting the amount of the free toxic factor could be represented best. The

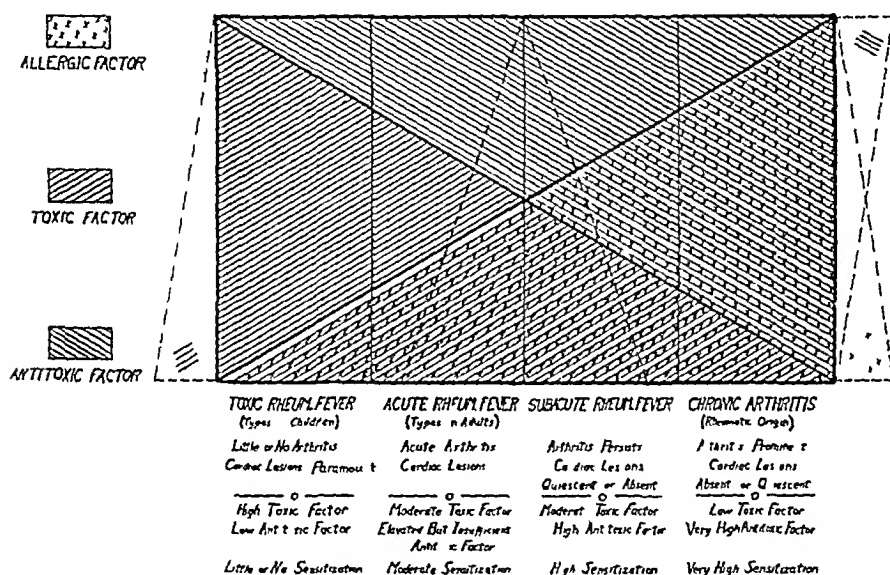


Fig 1—A graphic representation of the interrelation of the factors entering into the pathogenesis of rheumatic disease according to the hypothesis presented.

figure is divided into four vertical zones to illustrate in a general way the conditions of the hypothesis as applying to four types of clinical cases. The conditions applying to the individual case may be represented as those projecting on a vertical line through the figure. With these three factors operating as indicated in the hypotheses, it can be readily seen that a case as represented by a vertical line through the extreme left of the figure presents entirely different underlying conditions from one represented by a line through the extreme right, so that one might expect a difference in clinical manifestations as unlike as those of acute rheumatic carditis in children in the first instance and a low grade chronic atrophic arthritis in the latter. Yet each of these might arise primarily because of contact with the same streptococcus. The nonspecificity of the sensitization factor as to serologic type of streptococcus, however, does not limit the manifestations of chronic arthritis to a single specific streptococcus, whereas the manifestations of the acute rheu-

matic carditis are limited to a single specific streptococcus because of the biologic specificity of the toxic factor

The problem in the acute rheumatic carditis thus appears to be that of adequate neutralization of the toxic factor without transferring to the patient hypersensitization to the protein of the streptococcus. The problem as represented for the chronic arthritis is not that of increasing immune bodies which are already present in quantity sufficient to neutralize the small amount of the toxic factor as it is formed, but rather the elimination of the condition of hypersensitiveness which the patient has probably acquired by virtue of the building up of the existing immune bodies. In the former instance the indication is for antiserum, in the latter for minute 'desensitizing' doses of the bacterial protein as available in saline extracts of the bacteria.

Clinically we see acute rheumatic fever without severe arthritic involvements. This is common in the first attacks in early childhood nevertheless, children are especially susceptible to the destructive and proliferative lesions within the heart. The attack of acute rheumatic fever develops with an acute upper respiratory infection and the patient is sick and toxic for from three to five days in advance of the onset of the arthritic involvements. During this period cardiac damage begins before the joint lesions appear. This early phase represents, or the disease in children who develop no joint symptoms, represents the purely toxic stage of the disease. This is the condition which I hold to be due to a specific toxic factor which may be neutralized by its specific antiserum. According to this conception, the appearance of acute arthritis is a favorable sign, not only for pointing out the clinical diagnosis, but it is to be regarded also as indicative that the patient is beginning to build up his immune bodies.

In patients treated with the antiserum of *Streptococcus cardioarthritidis* the prompt clearing up of the appearances of a severe clinical toxemia is an outstanding feature. A second feature of great importance is what I have termed the 'focal' reaction following antiserum. This arises regularly when large doses of the antiserum are administered and manifests itself by the prompt appearance of new joints involved in an acute arthritis. For some reason this arthritis almost always affects the small joints of the hands. With this reaction large joints primarily involved usually clear up promptly. Untreated, the joints involved with the focal reaction subside in two or three days. Salicylate administration affects them favorably. Salicylate administration before administering antiserum, tends to prevent these focal reactions. During the focal reaction the patient's temperature remains elevated and fails to show the remissions which are common in the untreated case. The leucocyte count is elevated at times very greatly elevated. The skin loses its characteristic pallor, and sweating is not troublesome. The joints involved in the reaction are red and swollen but not particularly painful, and they are less tender than one would expect.

In our hypothesis of rheumatic fever this focal reaction represents the Arthus Phenomenon. The antiserum has supplied antitoxin, as well as antibodies of the protein factors of the streptococcus. The former detoxifies the patient and protects against further damage to the cardiac muscle. The lat

ter by uniting with the protein factor of the infecting streptococci provides the antigen-antibody complex of the dominant allergic factor in such amount as to aggravate the exudative lesions of the joints

In a practical way, this reaction (an exudative phenomenon) is ameliorated by giving the antiserum in small, divided doses, thus bringing about fractional combinations of antibody and antigen with the idea not only of reducing quantitatively the severity of the reaction, but also with the hope that such fractional production of the antigen-antibody complex will serve in partially desensitizing the patient against it. Or better still is the combining of the broken dose method with the use of moderate doses of salicylate by mouth

Another observation of practical importance is concerned with the reactions following the injection of the vaccine, or of the soluble products of *Streptococcus cardioarthritidis*. The soluble product in use is prepared by suspending the killed microorganisms, washed free of the liquid media in which they are grown, in normal saline in concentration of 100 million microorganisms per 1 c.c. After seven days in the refrigerator, the bacteria are removed by filtration. The soluble products are obtained in the saline menstruum. This is diluted with normal saline for use clinically. At first it was employed in dilutions of 1:10 and 1:100, later, as these dilutions proved too potent, weaker dilutions were prepared. At present dilutions of 1:100 millions, 1:10 millions and 1:1 million are used. It has required more than eighteen months of study to reach the present minute doses of this product. It is noteworthy that the starting dose in a course of treatment is now  $\frac{1}{20}$  c.c. of the 1:100 million dilution. Some conception of the minuteness of this dose is gained when one considers that this represents  $\frac{1}{20}$  of the soluble extract from one streptococcus. Based on nitrogen determinations on the undiluted extract, the initial dosage contains one-twenty billionth of a milligram of protein. Yet with such amounts definite focal reactions in the joints, and systemic reactions occur in acute rheumatic fever, as well as in chronic arthritis of atrophic and hypertrophic forms. We believe that, even if this means nothing by way of specific bacterial sensitization, it does represent one of the most outstanding examples of hypersusceptibility to a bacterial product thus far recorded in medical literature. In our scaling downward of the dosage, it was noted that the injections were followed by definite general and focal reactions in patients with chronic arthritis and that such patients lent themselves better to the study of these extracts than did patients with acute rheumatic fever in whom harm was likely to result from improperly adjusted dosage. With the larger doses these reactions came on slowly, requiring from three to five days to reach their height. As the latter small doses were approached it was noted that they came on more promptly, and at present definite reactions are noted with doses as small as  $\frac{1}{20}$  c.c. of the 1:100 million dilution, which come on as early as from three to eight hours after the injection. This tardy appearance of the reaction with the larger doses, and its prompt appearance with the small doses is an observation which cannot be definitely explained at present.

On the basis of the hypothesis offered above it can be accounted for in the following manner. The individuals showing these responses have some grade of immunity. The introduction of antigen into them permits the union of the antibody which they possess with this antigen, thus forming the antigen antibody complex to which they exhibit a hypersensitiveness. The symptoms of the reaction are in this conception a manifestation of the Arthus Phenomenon. But for the exhibition of the Arthus Phenomenon this union alone does not furnish the proper conditions. These two factors must be combined in the proper relative proportions. An excess either of antibody or of antigen in the complex prevents the appearance of this reaction. The larger doses of antigen introduced into patients furnish an excess of the antigen factor, so that the appearance of the reaction is delayed until this excess is destroyed or eliminated. With the very small doses used recently, proper conditions are at once established and this union of antigen and patient's antibody occurs in the proper relative proportions for the prompt manifestations of this reaction. Bringing these considerations back to the patient, these prompt general reactions following the small doses of antigen appear to be exudative phenomena in that they are ameliorated by the oral administration of salicylates. This further suggests that they arise from the dominant allergic factor, the antigen antibody complex of the protein antigens of bacteria, rather than from the toxin antitoxin complex. As such the protein antigens of various bacteria would give rise to them to a greater or less degree. Heating of the soluble extracts of the bacteria would not eliminate the reaction. Both of these conditions apply as will appear later under the discussion of chronic arthritis.

This working hypothesis might be pursued further to raise such questions as: Are these small doses of antigen sufficient to build up a permanent immunity? The evidence at present indicates that they may not be. Do they merely desensitize the patient? If they do, is it possible after desensitizing with small doses of antigen to practice the giving of a few large doses of vaccine to build permanent immunity, or whether the permanent immunity, if at all possible, may not have to be established by the use of the antigen antibody mixtures prepared *in vitro*? But these considerations while very important in the solution of the problem belong to the realm of the rapidly advancing science of immunology.

The manner of preparing the soluble antigen as well as the extremely small dosage tolerated by rheumatic patients and its apparent harmlessness to normal individuals suggest that it may be only the desensitizing agent. On the other hand its ability to precipitate relapses which exhibit both the exudative and the proliferative types of lesions in patients convalescing from rheumatic fever suggests that it contains all of the substances entering into the production of the disease. According to our hypothesis it might be presumed to have both the endotoxin and the various protein sensitizing antigens. Work which has just been started in which the antigen heated at 100° C. for two hours is employed in patients with chronic arthritis shows that definite desensitizing phenomena are obtained. This indicates that thermostable protein antigens are present, but our studies have not progressed far enough to



give definite indications that a thermolabile antigen has been eliminated from the product by the heating process

Our earlier use of vaccines in dosage equivalent to many times those represented by the antigen dilutions now used may have been more effective in inducing active immunity. Their use, however, was decidedly unsafe. It is not improbable that they may be employed effectively and with safety in patients desensitized by means of heated soluble antigens. The principles underlying the active immunization of patients against pathogenic streptococci, to some of the products of which they exhibit a hypersensitization, constitutes one of the greatest needs of present medical practice. Upon the development of these principles depend any advances which will be made in the specific prophylaxis of rheumatic fever with its appalling toll of cardiac disease.

In closing I must mention chronic arthritis, because our experience with the vaccines and antigens of *Streptococcus cardioarthritidis* has shown that there is a relationship between rheumatic fever and that group of inadequately classified arthritides commonly spoken of as atrophic, hypertrophic, rheumatoid and deforming arthritis. This relationship may, however, be more apparent than real. These diseases appear to be on a purely allergic basis, an allergy which may be that due largely to "protein specific" antigens common to streptococci, pneumococci and perhaps other microorganisms and in which species specific antigens may play a minor rôle. The fundamental problem in their treatment appears to be that of adequate desensitization. Since *Streptococcus cardioarthritidis* contains this nonspecific allergizing antigen its products become effective in this desensitizing process.

Saline extracts of *Streptococcus cardioarthritidis* have been used largely and effectively in the treatment of these cases. As mentioned above they still retain desensitizing properties after prolonged heating at 100° C., and it is probable that the heated product will eventually prove more practical than the unheated, which possibly may contain a subtle endotoxin. Our experience, however, thus far has been largely with the unheated product, and upon experience with it, the following statements are based. Improvement attends its use in this group of patients. This appears more striking and permanent where the closed foci harboring streptococci have been eliminated. Employed in excessive dosage, no improvement or actual activation of the disease results. Focal reactions in the joints, such as pain, stiffness and swelling are constant following injections. Occasionally redness of the skin over the acutely swollen joint follows the injection. Gastrointestinal symptoms, anorexia, nausea, vomiting, hyperperistalsis, also nervous and emotional symptoms, tremors, insomnia, irritability, melancholia, uncontrollable crying, etc., are frequently observed.

Soluble extract has been prepared from a number of strains of *Streptococcus viridans*, all of which had been isolated from blood cultures in subacute bacterial endocarditis. These have been prepared in the same manner as the soluble antigen of *Streptococcus cardioarthritidis* and used in similar dilutions in patients with chronic arthritis, i. e., 1:100 million and 1:10 million dilutions. With these extracts reactions similar to those obtained with the soluble antigen of *Streptococcus cardioarthritidis* are being obtained in these

patients This observation further strengthens the hypothesis that streptococci contain a common protein factor to which patients with arthritis are hypersensitive, and suggests that this hypersensitization is perhaps the basic underlying condition which maintains the chronicity of the arthritis The lack of streptococcus type specificity in this hypersensitivity is in accord with Swift's experimental work on bacterial allergy

From the standpoint of the treatment of the patient with chronic arthritis these observations have a particular significance because they suggest that we need not of necessity obtain the particular strain harbored by the patient for the preparation of a vaccine or a soluble extract to be used in treatment Practically any streptococcus contains the antigen to which the patient is hypersensitive and therefore stock preparations rather than autogenous ones may be used effectively This simplifies very much this form of treatment of chronic arthritis in two particulars First it avoids the isolation of streptococci and preparation of autogenous streptococcal products and second, it enables the use of a stock product which can be standardized more exactly as to dosage requirements This latter consideration is one of extreme importance because there appears to be a rather narrow margin of safety between the effective therapeutic dosage and harmful overdosage

For the adjustment of these dosage requirements to the patient's needs it has been found more practical to employ saline extracts of streptococci rather than vaccines While our practice of very minute dosage of the bacterial extracts is in line with the developments of Warren Clowe and of Burbank in the treatment of chronic arthritis with small doses of vaccine it utilizes dosage which is too small to be obtainable with vaccines e.g. the  $\frac{1}{20}$  of the soluble extract of a single streptococcus The use of the soluble extracts of streptococci therefore cannot be compared with that of minute doses of vaccine It is more strictly analogous to the pollen extracts used in hay fever or to tuberculin Since precautions are taken to prevent autolysis during the saline extraction of the bacteria these extracts are not of the nature of the various bacterial autolysates employed in therapy We believe therefore, that their preparation and use in minute dosage represents a refinement in immunotherapy and that clinical results are obtainable which would be impossible from the use of either bacterial vaccines or autolysates in dosages commonly employed

The employment of this form of therapy does not relieve the physician of the arduous task of identifying and eliminating focal infections One could not expect "desensitization" treatment to be effective in the presence of a closed "sensitizing" focus of infection, any more so than could he expect the desensitization treatment of hay fever from sensitization to ragweed pollen to be effective in a patient exposed to this pollen at the height of its season

#### SUMMARY

The role of streptococci in rheumatic disease is discussed, and an hypothesis embracing a dual nature of its pathogenesis is offered as follows

The specific toxin of a streptococcus is suggested as operative in the production of the "destructive" and "proliferative" types of lesions in rheu

matic fever, while the patient's hypersensitization to an allergen associated with the protein of streptococci is presented as concerned in producing the "exudative" lesions. The specificity of this sensitizing substance, or allergen is not dependent upon the type of streptococcus supplying it.

Chronic arthritis is presented as an allergic disease, the hypersensitive state of the patient being due to this allergen contained in streptococci without regard to a particular type.

The specific treatment of rheumatic fever embraces both the neutralization of the toxemia, and adequate desensitization.

The treatment of chronic arthritis is concerned largely with the elimination of foci harboring streptococci, and with adequate desensitization.

Stock extracts of streptococci may be employed in this desensitization, and because their dosage requirements may be better determined through extensive clinical application they are preferable to autogenous preparations.

#### REFERENCES

- 1 Poynton, F J Proc Bath Conference on Rheumatic Diseases, 1928, p 147
- 2 St Lawrence, W J A M A 79 2051, 1922
- 3 Faulkner, J M, and White, P D J A M A 83 425, 1924
- 4 Poynton, F J, and Paine, R Lancet 2 1900
- 5 Atwater, R M Am J Hyg 7 343, 1927
- 6 Coombs, C, Miller, R, and Kettle, E H Lancet 2 1209, 1912
- 7 de Vecchi, B Arch de Med Exper et d'Anat Path 24 352, 1912
- 8 Jackson, L J Infect Dis 12 364, 1913
- 9 Cecil, R L J Exper Med 24 739, 1916
- 10 Rosenow, E C J Infect Dis 14 61, 1914
- 11 Small, J C Am J Med Sc 173 101, 1927
- 12 Kreidler, W A J Infect Dis 43 415, 1928
- 13 Small, J C Am J Med Sc 175 638, 1928, Ann Int Med 1 1004, 1928, Bath Conference on Rheumatic Diseases, British M J, p 856, May 21, 1918, Atlantic M J 31 802, 1928
- 14 Bell, W P, Jodzis, F, and Fendrick, E Arch Path 6 812, 1928
- 15 Birkhaug, K E Soc Exper Biol & Med Proc 24 541, 1927, J Infect Dis 40 549, 1927
- 16 Hitchcock, C H J Exper Med 48 393 and 403, 1928
- 17 Barlow, Lazarus, P Bath Conference on Rheumatic Diseases, British M J, p 856, May 19, 1928
- 18 Annals Pickett Thompson Research Laboratory, Vol III, Plate 6, Figs 4 6, Plate 31, Figs 1 3, and Plate 34, Figs 4 6, also Vol IV, Part I, Plate 3, Figs 1 6, Plate 4, Figs 1 6 and Plate 6, Figs 1 13
- 19 Annals Pickett Thompson Research Laboratory 3 p 264
- 20 Lowenberg, H Klin Wchnschr 25 1170, 1928
- 21 Lowenberg, H Personal communication
- 22 Herry Bull Acad Roy Med Belg 28 Series 4, 76, 1914
- 23 Faber, H K J Exper Med 22 615, 1915
- 24 Andrews, C H, Derick, C L, and Swift, H F J Exper Med 44 35, 1926  
Derick, C L, and Swift, H F Proc Soc Exper Biol & Med 25 222, 1927  
Swift, H F, Derick, C L, and Hitchcock, C H J A M A 90 907, 1928  
Swift, H F, and Derick, C L Proc Soc Exper Biol & Med 25 224, 1927
- 25 Swift, H F, Derick, C L, and Hitchcock, C H J A M A 90 907, 1928
- 26 Zinsser, H, and Yu, H Arch Int Med 42 301, 1928
- 27 Dochez, A R, and Stevens, F A J Exper Med 46 487, 1927
- 28 Birkhaug, K E J Infect Dis 43 280, 1928
- 29 Von Dungern, F Zentralbl Bakteriell, Abt I 34 355, 1903
- 30 Irving Jones, E I M Arch Int Med 42 784, 1928
- 31 Arthus, M Compt Rend Soc de Biol p 718, 1903

# PRIMARY SARCOMA OF THE SPLEEN (RETICULUM CELLED)\*

## REPORT OF A CASE

By TASKER HOWARD M D, BROOKLYN N Y

PRIMARY sarcoma of the spleen is a rare disease Krumhhaar<sup>1</sup> encountered four cases in 6,500 autopsies Smith and Rusk found in the literature 104 cases of primary malignant disease of the spleen, all of which they considered sarcoma Their paper includes an abstract of most of these cases and they add a report of two additional cases to this list Since that time cases of primary sarcoma of the spleen have been reported by Ladreyt<sup>2</sup> (one) de Brun<sup>3</sup> (one), Beasley<sup>4</sup> (one) Birman<sup>5</sup> (one) Kohler<sup>7</sup> (one), Adam<sup>8</sup> (one) Eichenbusch<sup>9</sup> (one), and Krumhhaar<sup>1</sup> (four) The record of another example of this disease follows

*Case Report*—Miss M H seventy three years of age single a retired school teacher born in the United States was admitted to the Long Island College Hospital the first time November 8, 1926, complaining of fever diarrhea and weakness

The family history was irrelevant

The previous history included an attack of pneumonia as a child, and a tendency to an occasional digestive upset which apparently most often took the form of diarrhea She had been told twenty five years previously by Dr Jacob Fuhs that she had an achlorhydria Five years before admission she had a diarrhea which lasted the better part of the winter and was finally controlled after treatment in a hospital in California For several years she had been known to have a blood pressure which ran over 200 mm systolic most of the time

The onset of the present illness was dated by the patient as about six weeks before admission, when she was rather suddenly taken with diarrhea and fever while travelling in France At about the same time she noticed some abdominal soreness and a mass in her abdomen She was in bed several weeks before she was able to return home and kept in her bed on the steamer coming to the hospital within two days of landing Meantime she had grown very weak and had noticed some swelling of the legs The diarrhea had largely subsided though the bowels still tended to be a little loose She had never noted soreness of the tongue or mouth or numbness of the hands or feet She stated that she had been of a sallow color possibly a little yellow for a long time

On admission her temperature was 100.4 pulse 100 and respiration 26 She was rather poorly nourished, and sallow in color showing considerable pigmentation of the hands and face There was nothing significant in the examination of her head and neck except the absence of any evidence of glossitis or stomatitis and the absence of adenopathy

The chest was of the normal type and the lungs quite negative to examination The heart seemed enlarged and presented a 'squealing' systolic murmur which was audible all over the chest The arteries were definitely thickened and the blood pressure 180 mm systolic, 110 diastolic

In the abdomen a splenic tumor dominated the picture It extended 4 cm to the right of the midline and down to the iliac crest The notch could be readily felt The organ was smooth hard and not tender The liver edge was also palpable below the level of the umbilicus

From the Medical Service of the Long Island College Hospital  
Received for publication December 8 1928

The extremities showed no edema The knee jerks and ankle jerks were absent

#### The Laboratory Data

Urine 1015, albumin faint trace, few hyaline casts and leucocytes No bile present  
Urobilin was not looked for

Gastric analysis Achlorhydria, total acidity 12, otherwise not noteworthy

Blood Wassermann Negative

Blood						Large	
Date	Hb	W B C	R B C	Polys	monos	Lymphs	Eosins
11/ 8	50	3,200	2,550,000	62	26	12	0
11/12	48	2,800	2,496,000	54	28	16	0
11/18	49	2,200	2,600,000	61	24	14	0
11/23	53	3,200	2,700,000	68	13	17	2

The index in these counts is just about 1 No nucleated reds were seen There were some irregularities in the shape and size of the red cells, and some polychromasia was noted The high large mononuclear count is noteworthy The platelet count was 288,000

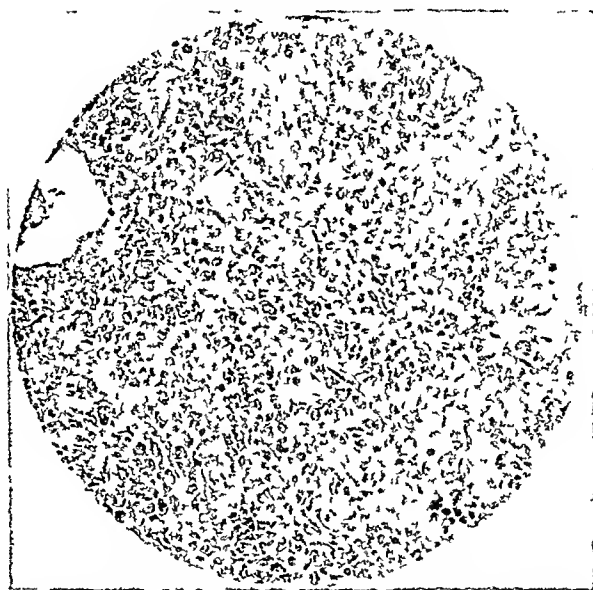


Fig 1—Reticulum celled sarcoma of the spleen The microphotographs were taken by Mr J V Dunn.

The fragility was found to be slightly less than that of a normal control Hemolysis started at 0.36 per cent and was complete at 0.27 per cent, the figures for the control being 0.40 per cent and 0.32 per cent Fifty reticulated cells per thousand were found by vital staining

The icterus index was 7 on November eighth and on November sixteenth it was 8

The van den Bergh reaction showed a delayed direct response and an immediate indirect response on two occasions

*Course of the Disease*—In the course of three weeks, with rest in bed, sunshine, and fresh air, hydrochloric acid and arsenic and iron, she made a great advance symptomatically, although it will be noted that there was but little change in the blood count She was allowed to go home, where she continued to gain, her hemoglobin reaching 76 per cent by the end of January Part of this period she was supposed to be upon the Minot and Murphy liver diet, although not until she had made the greater part of the gain ultimately attained It was difficult for her to follow the prescribed diet and she never entirely succeeded After January she began to lose ground, becoming paler and weaker, with a

gradual diminution in the blood count. By the middle of June she was obliged to return to the hospital, her hemoglobin having fallen to 46 per cent, her red cells being 3,072,000 and her leucocytes 3,200. At this time she developed an upper respiratory infection, to which she was quite prone. She exhibited an irregular fever and a tendency to excessive sweats. She gradually failed and died July 16, 1927. There was no particular change in laboratory findings or physical signs at the time of her second admission. During this period she was under the care and observation of Dr. George H. Roberts.

*Autopsy*—The spleen is enormously enlarged, weighing 1800 gm. The enlargement is regular so that the normal configuration of the organ is retained. At the hilus and along the lienal artery are enlarged and congested lymph nodes. A large part of the surface of the spleen is grey white in color suggesting extensive infarction. The remainder of the surface is of a dark brick red color. The consistency is slightly firmer than normal. Over the outer surface are found some recent adhesions. On section the normal splenic structure is entirely lacking, much of the tissue being replaced by an irregular caseous looking mass. Just below

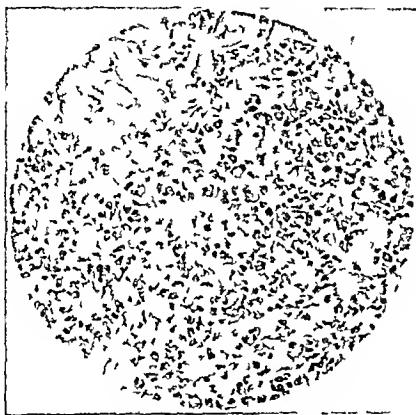


Fig. —Secondary invasion of the liver

the cortex this tissue is most abundant, accounting for the surface picture above noted. Between these areas the tissue is dark red in color and much of it is hemorrhagic.

The liver is large and pale.

Numerous large retroperitoneal lymph nodes are encountered which on section are uniform in structure and opaque.

*Microscopic*—Lymph Nodes. All of the enlarged lymph nodes show the same structure. The normal architecture is entirely replaced by a new growth. The cell type is that of a reticulum cell with round or ovoid vesicular nucleus and fairly abundant cytoplasm. Giant forms are occasionally encountered and numerous mitotic figures are seen. Among these cells are scattered varying numbers of lymphocytes and a delicate reticulum is present throughout.

*Spleen*. Much of the tissue is necrotic and a good deal of congestion is present throughout. In certain areas the cells are fairly well preserved and the structure is seen to be identical with that of the lymph nodes.

*Liver*. The new growth has invaded the liver and much of the parenchyma has been replaced. The appearance here is much the same as seen in the lymph nodes. The distribution of the tumor throughout the liver does not seem to be related to the anatomical structure.

of the organ, central and peripheral portions of the lobules being equally involved. The remaining liver cords show marked cloudy swelling.

*Diagnosis*—Reticulum celled sarcoma.

There are some points about the case as it presented itself clinically that are worth noting. In many ways pernicious anemia was strongly suggested, as in (1) the grave anemia with the high index, (2) leucopenia, (3) achlohydria, (4) high icterus index and indirect van den Bergh, (5) the apparent remission, which to the patient's mind was practically complete, and the relapse, and (6) the suggestion of cord involvement in the absent reflexes. However, the spleen was larger than occurs in this disease, and several other minor points, such as the absence of glossitis, megalocytosis, and thrombocytopenia, made this diagnosis unlikely. Chronic hemolytic jaundice could be ruled out

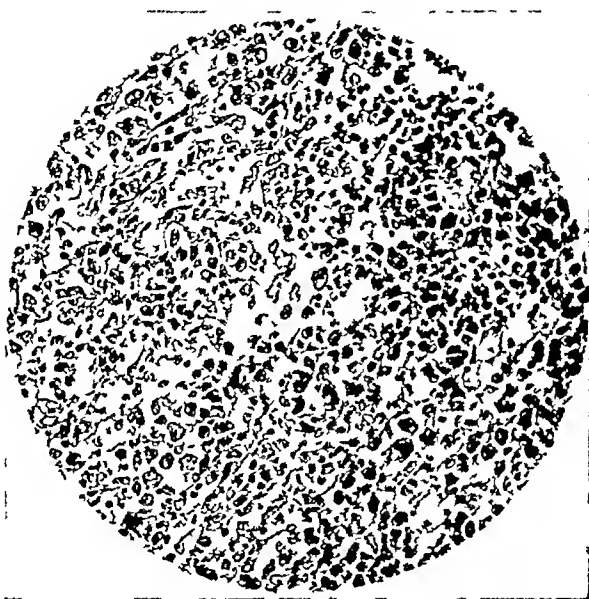


Fig. 3—The same tumor involving a lymph node

by the normal fragility of the erythrocytes. In Banti's disease the anemia is more characteristically of the secondary type, and evidence of active hemolysis is seldom as frank. The clinical diagnosis was hemolytic anemia with splenomegaly of undetermined character. Splenectomy was considered, but in view of the age of the patient, the condition of her vascular system and blood, the hepatic enlargement, and the severity of the operation, it was not performed. Splenectomy for primary sarcoma of the spleen has been performed successfully in 21 of 39 cases collected from the literature of Smith and Rusk.<sup>2</sup> Of the 18 who died, 7 succumbed to the operation and 11 to recurrences.

The type of anemia in these cases is of some interest. Blood counts are available in 25 of the cases cited. There was some degree of anemia in all but one and a high degree in a number of them. The color index was reduced in about half of them and was not reduced in half. The increase in large mononuclear cells noted in this case was not reported in any of the others.

Fragility was tested in but very few cases, and as in this case it proved normal when tested

No record was found of any studies of the bilirubin by means of the icterus index or the van den Berg test. The observations in our case seem to indicate a pathologic degree of hemolysis

#### SUMMARY

To the 115 cases of primary sarcoma of the spleen found in the literature is added the report of an additional case which clinically strongly resembled pernicious anemia

#### REFERENCES

- 1 Krumbhaar E B Ann Clin Med 5 9 833, March 1927
- 2 Smith and Rusk Arch Surg 7 371 1923
- 3 Ladreyt F Bull Acad de Med 99 118 July 31, 1923
- 4 de Brun New York M J 118 8, July 18 1923
- 5 Beasley B T J M A Georgia 13 305 September 1924
- 6 Birman, A Wien klin Wchnschr 57 1237 Nov 27 1924
- 7 Kohler, H Arch f klin Chir 136 194 1924
- 8 Adam, L Zentralbl f Chir 55 481 Feb 25 1928
- 9 Eichenbusch F Frankfurt Ztschr f Path 35 464, 1927

### THE EFFECT OF MERCURY AND SODIUM OXALATE ON BLOOD CALCIUM\*

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THIS series of experiments the first of which is here reported, was undertaken to ascertain the effects of certain drugs upon the level of calcium in the blood

Various investigators have reported that mercury changes the calcium metabolism of the body. Chari<sup>1</sup> found that the calcium was diminished in the vascular elements, cells and nerves of the intestinal wall, and Koemiger<sup>2</sup> cited by Biberfeld, stated that the calcium content of the blood almost never increased, as a result of the administration of mercury, in acute experiments. Klemperer<sup>3</sup> and Schmiedeberg,<sup>4</sup> however, report that there is no modification in the calcium of the blood as a result of the administration of mercury.

That the metabolism of calcium in the body is not influenced by mercury, in spite of the presence of crystals of calcium phosphate in the kidney, is stated by Heilborn,<sup>5</sup> Bmet<sup>6</sup> Salkowski, Kaufmann, and Prevost and Eternod.<sup>7</sup> Several authors, Heilborn,<sup>5</sup> Sabbatani,<sup>8</sup> Jablonowski,<sup>9</sup> Prevost and Furbringer,<sup>10</sup> and Senger<sup>10</sup> report that mercury has a decalcifying action upon the osseous tissues, which become fragile and poor in lime.

The determinations were made upon animals in which the toxic effects of mercury were being investigated. This was done at the suggestion of Dr Salant (personal communication) who thought there might be some causal

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relationship between the toxicity of mercury and the calcium content of the blood, since the action of mercury upon the heart in many of his experiments was suggestive of a disturbance of the normal calcium-potassium ratio

#### EXPERIMENTAL METHOD

Healthy cats were used under methane anesthesia, with the exception of one experiment, in which ether was used. The animals were tracheotomized, both femoral veins, and the right carotid artery cannulated, the former for the administration of the drug, the latter for the registration of blood pressure, respiration also being recorded.

Mercury succinate was injected into the femoral vein at the rate of one milligram of mercury per kilogram of body weight at intervals of fifteen minutes, until sufficient mercury had been administered to cause death. Blood was taken from the carotid artery for the control determination, the final sample being obtained from the heart immediately after death. In those experiments in which only normal blood was analyzed, samples were drawn directly from the heart. Blood calcium was determined by the method of Roe and Kahn,<sup>11</sup> all determinations being made in triplicate.

The blood calcium was studied in animals on different diets, and determinations were made before and after the administration of mercury, in the majority of the experiments.

#### RESULTS

It will be seen by examination of Table I that animals fed upon a diet consisting entirely of salmon had a higher control level of blood calcium than did those fed solely upon beef, or those which had been starved for seven or eight days. The lowest initial calcium reading was obtained from an animal brought in off the street and fed chopped beef for five days. The calcium

TABLE I  
EFFECT OF MERCURY ON BLOOD CALCIUM

EXPERIMENT NUMBER	MG CALCIUM PER 100 CC BLOOD CONTROL	LETHAL DOSE MERCURY IN MG PER KG	MG CALCIUM PER 100 CC AFTER HG	DIET
690	9.80			Bread and Salmon
691	8.71			Bread and Salmon
692	8.59			Bread and Salmon
693	9.27			Bread and Salmon
714	8.62			Bread and Salmon
715	9.68			Bread and Salmon
694	11.70	12	11.38	Salmon
699	13.78	14	9.89	Salmon
700	11.06	18	8.87	Salmon
702	11.26	16	9.29	Salmon
706	9.90	5	10.10	Bread and Salmon
707	8.08	11	7.06	Bread and Salmon
716	9.78	24	7.17	Starved 7 days
717	8.17	10	8.45	Starved 8 days
719	9.51	14	6.97	Beef 3 days
720	9.51	11	7.26	Beef 4 days
721	9.52	7	7.74	Beef 2 days
722	8.42	6	7.27	Beef 5 days
723	7.85	13	4.94	Beef 5 days
724	8.98	10	7.18	Beef 8 days

level of the remainder of the heef fed cats was little different from that of the animals which had been starved. However, the duration of the heef feeding period was probably too short to warrant definite conclusions being drawn.

The average control level of calcium in the entire series of animals was 9.69 mg, whereas that of 5 salmon fed cats was 12.20 mg per 100 c.c. of blood. The average of the heef fed and starved animals was the lowest of the series namely, 8.97 mg, and those fed a diet consisting largely of bread, supplemented by small quantities of salmon averaged 9.11 mg of calcium per 100 c.c. of blood.

Mercury in amounts ranging from 5 to 25 mg per kilogram of body weight, produced a rather marked diminution of blood calcium in 13 of the 16 experiments. Animals 706 and 717 show an apparent increase, which, however, does not greatly exceed the limit of experimental error of the method and number 694 shows a decrease of about the same magnitude.

Sarionat and Roubier<sup>1</sup> report 5 experiments on guinea pigs, two being used for controls in which the calcium of the skeleton and soft parts of the body were determined. Three were injected subcutaneously with sodium oxalate until death occurred, the average amount required being 0.195 gm for animals of average weight. Skeletal calcium was decreased from 0.496 to 0.378 gm, or approximately 24 per cent, and the soft parts from 0.179 to 0.101 gm, or 42.5 per cent per gram of ash.

In the determination of the mineral metabolism of a rabbit receiving 4 c.c. of 3 per cent sodium oxalate for three days, Luthlen<sup>2</sup> found an increased elimination of potassium and calcium, the former being greatest. Little change in magnesium and a retention of sodium.

To determine whether depletion of the blood calcium before the administration of mercury would have a different effect on the action of mercury, some animals were injected subcutaneously with sodium oxalate at the rate of 25 milligrams per kilogram of body weight, for periods varying from five to nine days. The results of these experiments appear in Table II. In those animals injected for the shorter period, five days, the initial calcium level before oxalate, was near the level of the entire control group. Three animals Nos. 709, 710, and 715, which were injected for nine days and one, No. 714 injected for eight days had a definitely low calcium content. The administration of mercury to these animals was followed in five of the six animals by the further reduction of the blood calcium while one No. 701 was unaffected.

TABLE II  
SODIUM OXALATE SUBCUTANEOUSLY FOLLOWED BY MERCURY

EXPERIMENT NUMBER	MG CALCIUM PER 100 C.C. BLOOD CONTROL	LETHAL DOSE HG IN NO PER KG	MG CALCIUM PER 100 C.C. AFTER NO	NUMBER DAYS INJECTED WITH Na <sub>2</sub> CO <sub>3</sub>
701	9.90	5	10.10	5
704	8.08	11	7.06	5
709	4.50	5	Too dilute to read	9
710	4.33	5	Too dilute to read	9
714	6.30	9	4.90	8
715	6.27	11	4.62	9

The question of variation of the calcium content of the blood as related to diet cannot be discussed, since no analyses of food intake and excreta were made. The average calcium content of the foods used, determined as calcium oxide, according to the tables of Sherman,<sup>14</sup> is as follows: Baker's bread 0.03 per cent, salmon 0.011 per cent, lean brisket 0.011 per cent, based on the edible portion. Total ash, from tables by Atwater and Bryant,<sup>16</sup> baker's bread 0.8 per cent, canned salmon 2.0 per cent, lean brisket 1.0 per cent. The figure of calcium content of salmon (Sherman) does not take into consideration the bone present in canned salmon, which would necessarily increase the percentage of calcium.

Possibly the fact that the cats fed exclusively on canned salmon shows a higher control level of calcium than the remainder may be in part explained by the statement that the coefficient of digestibility of salmon is about 12 per cent higher than that of bread (Sherman), and that when the diet was composed primarily of bread, peristalsis was probably greater. More calcium may have been lost in the excreta, and possibly less absorbed as a result of increased peristalsis, than in the case of the salmon fed animals.

#### SUMMARY

The administration of mercury succinate in acute experiments effects a considerable diminution of the calcium of the blood.

Blood calcium is diminished by the administration of sodium oxalate. Mercuric succinate administered to animals with low blood calcium results in a further depletion of serum calcium.

#### REFERENCES

- 1 Chauri, R. Arch f exper Path u Pharmacol 63 434, 1910
- 2 Koeniger. Diss Wurzburg, 1888. Cited by Biberfeld, J. Erg de Physiol 12 32, 1912
- 3 Klemperer, F. Virchow's Arch f Anat u Physiol 118 445, 1889
- 4 Schmiedberg, O. Pharmakologie Leipzig, Vogel
- 5 Heilborn, M. Arch f exper Path u Pharmacol 8 361, 1878
- 6 Binet, P. Revue med de la Suisse Rom 11 165, 1891
- 7 Cited by Tiffeneau, M. Dict de Physiol Tome 10, p 707
- 8 Sabbatini, L. Ann di Ehim e di Farm Milano 23 49, 1896
- 9 Jablonowski, G. Ueber die Einwirkung des Hg auf den Thierischen Organismus Berlin, 1885, O. Lange
- 10 Cited by Tiffeneau, M. loc cit
- 11 Roe and Kahn. J Biol Chem 67 585, 1926
- 12 Sarvonat and Roubier. Compt rend Soc Biol 71 104, 1911
- 13 Luxthlen, F. Arch f exper Path u Pharmacol, Leipzig 69 375, 1912
- 14 Sherman, W. H. Chemistry of Food and Nutrition, 1912, Macmillan
- 15 Atwater, W. O., and Bryant, A. P. U. S. Dept of Agriculture Bull 28, Revised, 1906.

# STUDIES IN THE ALIMENTARY TRACT OF MAN\*

## II STABILIZATION OF GASTRIC REACTION PATTERNS

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### INTRODUCTION

IN A previous article we set forth our conviction that the fundamental necessity for a study of motility in the human alimentary tract is the employment of stomachs (or intestines) of subjects carefully trained in roentgenology so that subconscious influences which modify the alimentary responses may be reduced to a minimum. In the present paper we propose to examine this thesis further as a preliminary to the development of a technique of diagnostic meals. Actually for the moment, this resolves itself into a consideration of the physical condition of the stomach.

In clinical writings the terms gastric tone and gastric peristalsis appear to indicate a distinction between these two forms of gastric activity, and, indeed, one form may be so obtrusive that it eclipses the other. Both however are evidences of smooth muscle irritability. In a roentgenologic investigation they express themselves in area and shape of shadow and in the character of the gastric movements.

### THE EMPTY STOMACH

On two different occasions (Sophomores Nos 314 and 104) we were fortunate enough to see on the fluoroscope the actual size of the empty stomach of the students before the barium meal was given. The gastric tube seemed to be reduced both laterally and anteroposteriorly, to the width and thickness of two fingers, it appeared about five to six inches in vertical height. We handed the prepared barium meal to the student and asked him to hold it for a few minutes. Meanwhile we examined carefully the delicate stomach shadow on the fluoroscopic screen. We observed that the stomach shadow widened out and lengthened during this period as though it were becoming filled with gastric juice. This change in outline was completed within thirty seconds, we were unable to obtain measurements. It may be that the very delicate outline is responsible for our failure to observe peristalsis or indeed any movement. But it would be natural at least to expect that in a condition of emptiness gastric movements would be less vigorous than when contents are present.

Since the above paragraph was penned we have had the opportunity of examining the same phenomena on several further occasions, always with the same result.

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## TYPES OF FILLING

The precise method of entry of barium into the stomach varies and indeed the same stomach may show different types of filling at successive examinations. Nevertheless the number of methods of filling was greater when we first commenced our study than it is today and it is quite possible that increasing control over our subjects, as the tradition of routine examination becomes established, may result in a reduction to order of what is at present a rather chaotic medley of observations.

The development of the barium shadow may take place from above downward or from below upward. Of the first type there are four methods. In the first the barium spreads itself instantly throughout the cardiac sac. It then either passes immediately in a continuous mass through the gastric tube to the pylorus or it sifts gradually through gastric fluid, rapidly secreted into the gastric tube, to settle more slowly in the pyloric vestibule. Secondly, it may be so held up in the cardiac sac as to give the appearance of an inverted barium cone from which a pencil-like stream trickles through the gastric tube to the pyloric vestibule. Thirdly it may outline the greater curvature only by a stream which however gradually broadens until, in some examinations, the lesser curvature is also outlined. In these three methods the essential principle is a filling of the cardiac sac with immediate or delayed passage to the pylorus, the time taken depending upon promptitude of relaxation of gastric musculature or speed of secretion of gastric juice. In the fourth and rarest method there is no spreading out in the cardiac sac but the barium passes down the lesser curvature, its shadow gradually widening (probably through gastric juice) until the greater curvature is also outlined.

There is a totally different method of filling in which it is difficult to follow the passage of barium from the cardia through the gastric tube unless, as sometimes happens, it defines the lesser curvature in its passage. The barium first collects in recognizable amount at the lowest part of the pyloric vestibule which then fills from below until there is a heavy shadow of barium up into the gastric tube. This type of filling occurs more frequently in the elongated, vertically placed stomach. We were at first inclined to associate it with Freshmen only but Sophomores exhibit it also. In Sophomores indeed we have observed quite frequently the faint unoccupied outlines of greater and lesser curvatures and a filling from above by the barium stream which passes gradually down the gastric tube, midway between the curvatures, to the pyloric vestibule. A faint barium shadow outlines the curvatures and shows that the greater is opening out laterally. Since the Sophomore stomach is more constant in its responses than that of the Freshman we are inclined to believe that this method of filling should be considered the most natural one.

## CHARTS OF THE GASTRIC FEATURES

In Chart 1 are represented the positions of cardia, pylorus, and greater curvature in all students examined between January, 1925, and October, 1927. The records are all from buttermilk meals, 16 ounces to the meal, except for the Sophomores in October, 1927, who had 5-ounce meals and whose record is listed separately. The total Freshmen are 86, the Sophomores somewhat less

through academic casualties. For each group the mode is easily recognized, the median is encircled and the mean position is designated by the letter *M*. Crude averages in series of such a dispersal do not inspire much confidence and indeed experience assures us that a difference of one unit is probably negligible, though an interval of two or more units is almost certainly significant. From this chart there seems to be no change in position of cardia or pylorus connected with duration of digestion or status of student. On the other hand greater curvature though showing no change associated with duration of digestion or size of meal undergoes an elevation in site in the change from Freshman to Sophomore. Small as it is the change noted in the chart is of im-

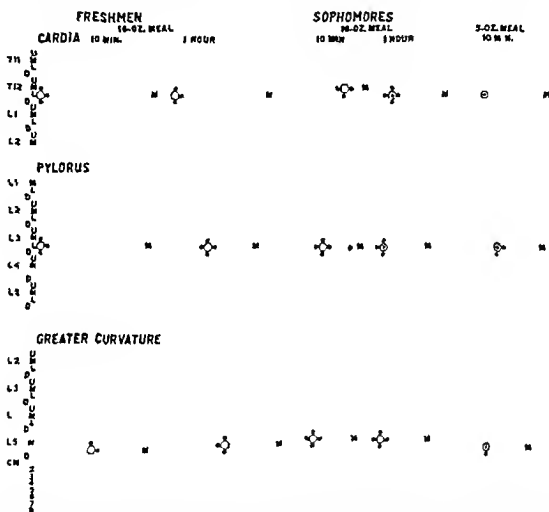


Chart 1—Relation of stomach levels to student status, period of digestion and size of meal. Vertebrae indicated in thirds upper middle and lower. Disc between fourth and fifth lumbar vertebrae also plotted. Median (encircled), mean (*M*) and mode all indicated.

portance. It was the first indication we had of the group response discussed in our last paper. It encouraged us to examine our individual records in detail and so discover the striking change which takes place in vertical dimension of the stomach with the progress of training.

With the data upon which the chart is made we have developed the summarizing Table I which especially in the common averages, shows the rise of greater curvature from Freshman to Sophomore. Greater clarity could not be expected from distributions with so large a range. Hence the full expression and meaning of the differences can be determined only by examination of the individual records. This has been done and attention, in consequence, focused on shadow area but before the results of this work can be properly

presented we must have further assurance regarding the dependability of technical methods involved

TABLE I  
GROUP AVERAGE STOMACH POSITION

		CARDIA		PYLORUS		GREATER CURVATURE	
		10 MIN	1 HR	10 MIN	1 HR	10 MIN	1 HR
Group I							
Freshmen	Jan 1925	LT12	MT12	LL3	LL3	LL5	UL5
Sophomores	Oct 1925	MT12	LT12	ML3	UL3	UL5	dL4 5
Group II							
Freshmen	Oct 1925	LT12	UL 1	LL3	dL3 4	LL5	LL5
Sophomores	Oct 1926	MT12	LT12	LL3	LL3	UL5	UL5
Group III							
Freshmen	Oct 1926	MT12	MT12	ML3	ML3	LL5	ML5
Sophomores	Oct 1927	LT12		LL3		ML5	
Common Average Stomach Position							
All Freshmen		LT12	LT12	LL3	LL3	LL5	ML5
All Sophomores		MT12	LT12	LL3	LL3	UL5	UL5

#### PRACTICABILITY OF RECORDING CHANGE IN GASTRIC DIMENSIONS

To test the precision with which it is possible to determine gastric dimensions we have made two series of observations on width, height, and area of stomach shadow. The results are given in Table II.

TABLE II  
CHECK RECORDS OF STOMACH DIMENSIONS

1 Width and Height				
	NUMBER OF STUDENTS	TRIAL	AV 10 MIN WIDTH	AV 10 MIN HEIGHT
Group I				
(Freshmen)	29	1	81.4 mm	251.2 mm
		2	80.8	249.6
Group II				
(Sophomores)	31	1	51.4	210.7
		2	50.5	211.0
2 Area				
	NUMBER OF STUDENTS	AVERAGE AREA 1ST TRIAL	AVERAGE AREA 2ND TRIAL	
Group I				
(Sophomores Indoor Occupation)	8	11797 sq mm	11554 sq mm	
Group II				
(Sophomores Outdoor Occupation)	9	12287	13031	

Width of shadow was determined at the lower limit of the Magenblase, a site which by experience we know to be relatively stable. Vertical height is the maximum distance between highest and lowest points of gastric outline measured parallel to the vertebral axis. Area is obtained by planimeter as described in our former article.

In the series for width and height the first group consists of 29 Freshmen with 16-ounce buttermilk meals. The second group consists of 31 Sophomores with 5-ounce sweet milk meals. Due care was taken to confuse the memory

of the observer who made the determinations. The area determinations were made on Sophomore students with 5 ounce meals.

Although the results of this trial are more uniform for linear dimensions than for area as might be expected our findings in all measurements justify us in proceeding with the investigation.

#### DIMENSIONS OF SHADOW ON REPEATED EXAMINATION

The qualitative difference in gastric response of Freshmen and Sophomores has been our main theme both in this article and our former one. Table III shows the gastric reactions in terms of three measurable dimensions: width, height, and area.

TABLE III  
RELATION OF STOMACH DIMENSIONS TO REPEATED EXAMINATIONS

1 Width				
	DATE	AV FRESHMEN 19 MIN WIDTH	DATE	AV SOPHOMORES 19 MIN WIDTH
Class of 1928	Jan 1925	711 mm	Oct 1925	723 mm
Class of 1929	Oct 1925	897	Oct 1926	831
Class of 1939	Oct 1926	811		
Av Freshman width 776 mm			Av Sophomore width 777 mm	
2 Height				
	DATE	AV FRESHMEN 10 MIN HEIGHT	DATE	AV SOPHOMORES 19 MIN HEIGHT
Class of 1928	Jan 1925	2521 mm	Oct 1925	2306 mm
Class of 1929	Oct 1925	2454	Oct 1926	2315
Class of 1939	Oct 1926	2594		
Av Freshman height 2493 mm			Av Sophomore height 2319 mm	
3 Area				
	DATE	AV FRESHMEN 10 MIN AREA	DATE	AV SOPHOMORES 10 MIN AREA
Class of 1928	Jan 1925	21545 sq mm	Oct 1925	18163 sq mm
Class of 1929	Oct 1925	22829		
	Feb 1926	22909	Oct 1926	29459
Class of 1930	Oct 1926	22999		
Av Freshmen area 22568 sq mm			Av Sophomore area 19396 sq mm	

From the Table it is apparent that the stomachs of the 1928 class were rather narrow, but comparing Freshmen and Sophomore records, initial width is a function of the amount of stomach contents and is not particularly influenced by other conditions. Length and area however are affected by some factor other than mere size of meal. The reduction in height between Freshman and Sophomore stages is about 20 mm. The average area is reduced from 22600 to 19300 sq mm amounting almost to one sixth of the latter figure. We have here a quite remarkable confirmation of the thesis presented in our first article, namely the relatively small size of gastric shadow in Sophomores, after ingestion of a standard barium meal, when compared with the findings recorded for Freshmen.

#### GASTRIC DIMENSIONS AND PERIOD OF DIGESTION

Since the faint shadow of the empty stomach cannot be measured on the rare occasions when it can be seen in fluoroscopic examination the nearest approach we can make to a comparison of dimensions in well filled and approxi-



mately empty states is an examination of the dimensions taken from radio grams obtained respectively ten and sixty minutes after ingestion of the meal. These results are given in Table IV. The sixty-minute stomach is not empty. In a normal healthy subject we often find that a remnant of barium contents is still present in the most dependent portion of the pyloric vestibule even six hours after a meal. This is expelled immediately upon or very shortly after the appearance of a new meal in the stomach.

The successive classes of students are arranged as groups, and a careful examination of the respective reductions in dimension shows a progression in which the least reduction is exhibited throughout in the October Freshmen. The reduction becomes more marked in the January (or February) Freshmen and is at its greatest in the October Sophomores. Further, Group III as Freshmen gives a better showing than Group I as Freshmen. A factor which we have defined formerly as the group response is at work increasing the vigor of reduction in all dimensions, even width. If we assume that this reduction in dimension is due simply to emptying, we still have to cope with the problem of how it comes about that emptying is more vigorous in Sophomores than in Freshmen and also in successive groups of Freshmen. We shall see later that the explanation is probably not a simple one of emptying rate.

#### RELATION OF GASTRIC DIMENSIONS TO SIZE OF MEAL

In Tables III, IV are records of dimensions obtained from 16-ounce butter-milk meals. Table V gives records from 5-ounce sweet milk meals in addition to embodying the averages obtained from the two previous tables.

TABLE IV

#### RELATION OF STOMACH DIMENSIONS TO PERIOD OF DIGESTION

1 Width					
		YEAR	AVERAGE 10 MIN WIDTH		AVERAGE REDUCTION FROM 10 TO 60 MIN
Group I	Freshmen	Jan 1925	71.1 mm		15.1 mm
	Sophomores	Oct 1925	72.3		17.4
Group II	Freshmen	Oct 1925	80.7		13.3
	Sophomores	Oct 1926	83.1		24.3
Group III	Freshmen	Oct 1926	81.1		17.7
	Total average reduction				17.6 mm
2 Height					
		YEAR	AVERAGE 10 MIN HEIGHT		AV REDUCTION 10 TO 60 MIN
Group I	Freshmen	Jan 1925	252.1 mm		22.1 mm
	Sophomores	Oct 1925	230.6		20.8
Group II	Freshmen	Oct 1925	245.4		10.0
	Sophomores	Oct 1926	231.5		19.1
Group III	Freshmen	Oct 1926	250.4		19.2
	Total average reduction				18.2 mm
3 Area					
		YEAR	AV AREA 10 MIN	AV AREA 1 HR	REDUCTION
Group I	Freshmen	Jan 1925	21545 sq mm	17343 sq mm	4202 sq mm
	Sophomores	Oct 1925	18163	13721	4442
Group II	Freshmen	Oct 1925	22829	18913	3916
	Freshmen	Feb 1926	22900	18077	4823
	Sophomores	Oct 1926	20450	14690	5760
	Total average reduction				4629 sq mm

TABLE V  
RELATION OF STOMACH DIMENSIONS TO SIZE OF MEAL

	16 OUNCE MEALS		5 OUNCE MEALS
	FRESHMEN 10 MIN AVERAGE	SOPHOMORES 10 MIN AVERAGE	SOPHOMORES 10 MIN AVERAGE
Width	77.6 mm	77.7 mm	51.4 mm
Height	249.3	231.0	210.7
Area	22458.0 sq mm	19306.0 sq mm	12756.0 sq mm

The initial shadow width which was 77.6 mm on the average in the Freshman 16 ounce meal, is still the same (77.7 mm) in the Sophomore meal of the same size. But it is only 51.4 mm in the Sophomore 5 ounce average. This suggests again what we have already intimated namely, that width bears initially a definite relation to stomach contents and is constant for a given size of meal.

We have shown that this is not true of height (Table III) and the reduction in height of shadow during the hour averages about 15 mm for October Freshmen and 20 mm for Sophomores (Table IV). The fluoroscopic records of these examinations usually state that at the end of the hour there appeared to remain about a quarter or rather less of the original contents. This was estimated on surface area of shadow only. The lateral fluoroscopic examination gives a very indefinite idea of anteroposterior dimension, though indeed this does not apparently change. Now the average reduction in width for Sophomores (Table IV) is about 21 mm. This subtracted from 78 mm gives the figure 57 mm which is strikingly near the new 5 ounce width, namely, 51.4 mm. Likewise the initial figure for Sophomore height reduced after an hour by 20 mm (Table IV) gives a final figure of 210 mm. The actual comparable figure for the 5 ounce stomach is 211 mm.

In the problem of area we find a similar result. The average Sophomore stomach shadow of 19,300 sq mm is reduced by 5100 sq mm during one hour and is hence brought to a final average of 14,200 sq mm. Now the initial area of the 5 ounce shadow approaches 13,000 sq mm.

It follows that our estimate of the amount of contents still present an hour after swallowing a 16 ounce meal is probably fairly accurate. The most instructive feature of these observations is that, worked out on the Sophomore stomach, all figures are quite logical and harmonious and their relationship to the actual contents seems assured. Hence as the Sophomore figures fall into line so perfectly there must be some influence, hitherto unrecognized, at work in the Freshmen records, throwing them into confusion. One might put it crudely by stating that the Freshman stomach is too large for its contents but that this initial overrelaxation becomes progressively less marked in succeeding examinations of a single group, as it does also in the examination of successive groups. Naturally we do not seriously mean to put forward any such absurd explanation. But we would draw attention to the influence upon gastric tone of another factor, of psychologic or rather of central origin, which complicates the normal reflex gastric responses. In the various phases of our investigation we are constantly meeting the same phenomenon which baffles all our efforts to obtain a standard reaction pattern in the stomachs.

of our Freshmen, but of which the influence is so greatly reduced in our Sophomore records that it is comparatively negligible. It is an inhibiting influence which limits the vigor and fullness of response in the untrained stomach.

#### THE VERTICAL DIMENSION OF THE MAGENBLASE

At this juncture we must return to the problem of vertical diameter of the stomach shadow. We have discussed rather fully the reduction in height of shadow which takes place during the hour following a meal and in the comparison of records taken from Freshmen and Sophomores. But we also must admit that this reduction finds no necessary or proportional counterpart in rise of greater curvature. For a long time we were at a loss to account for this. In the course of time we came to study the Magenblase itself and found that here also considerable change in vertical dimension takes place. When a meal is swallowed the elevation of the Magenblase can be clearly seen, the cupola of the diaphragm rising with it. The base of the Magenblase, which we have picked out as a stable level for measurement of width, however, remains constant in site. In some of our subjects we have found the vertical increase in Magenblase very marked, associated with extreme elevation of the diaphragmatic cupola. In all stomachs the reduction in Magenblase height goes on apace after some twenty minutes and at the end of an hour the reduction is quite pronounced. Many instances of reduction in gastric length were missed in our earlier work because our attention was focused on the greater curvature which scarcely changed in position, the contraction being almost confined to the fundic end. The slight respiratory distress which sometimes occurs during the first twenty minutes after a meal is probably associated with this phenomenon.

Vertical distension of the Magenblase can be observed on the fluoroscopic screen. It is evidently produced by a rapid accumulation of gas. In some subjects this occurs even before the actual swallowing of the barium meal. No matter whether it precedes, accompanies or follows the ingestion of the meal we have been quite unable to observe progress of gas down the esophagus. We also have looked in vain for gas entering the stomach through the pylorus. We are therefore forced to the conclusion that gas is formed in the Magenblase itself. And this phenomenon offers a problem for future elucidation. It is apparently analogous to the sudden accumulation of gas in the colonic flexures but is not so easily explained.

#### STABILIZATION OF THE STOMACH

At his first roentgenoscopic examination the Freshman shows certain signs of distress such as pallor, flushing, dryness of mouth, difficulty in speech, a cold clammy skin, perspiring palms, and jerky nervous physical movements. The manifestation of this distress varies in range and in degree, but with these outward signs we may expect others, more obscure, exhibited in the viscera. We have indeed already given reasons for the conclusion that such manifestations are to be found in the alimentary tract. The Sophomore, with his growing experience of medical training, rarely exhibits outward indica-

tions of distress. Nevertheless, even in him, an unexpected stimulus produces an immediate effect which, despite rapid recovery, points toward the condition characteristically found and more pronounced in the Freshman.

Recognizing then the disturbing effect of these emotional factors it has been possible to develop a method of training and a technique of observation by which the influence of these factors is minimized. Repeated examinations of the same subject are made under carefully controlled conditions and a rigid discipline. Not only is the subject under constant scrutiny but the discipline extends to examiners and observers who must work in perfect harmony. If even the slightest antagonism develops, of which the subject himself is entirely unconscious, it will make its effect evident in the experimental result.

A subject of even temperament exhibits a stomach in which there are but slight fluctuations in position and activity at successive examinations. We have called this equable type a tolerant stomach.

Few individuals exhibit a tolerant stomach at the first examination. In nearly all the stomach shadow controlled by measurements at later examinations, is long and broad. The peristalsis is feeble and often temporarily inhibited. Reduction in size of shadow and increased vigor of peristalsis are shown at successive examinations until after practice the responses to a constant stimulus are stabilized both in dimensions and in motility. Such a stomach we hold to exhibit evidence of the distress complex. According as the behavior pattern is further removed from the modal response, we term the degree, disquiet, apprehensive or distressed. In fear, the response reaches its culmination: there is then great exaggeration of shadow dimensions with complete inhibition of peristalsis. We have reason to believe that fatigue also induces, though in less degree, a somewhat similar behavior pattern.

On the other hand a subject, even though trained, may present the picture of an especially active stomach. The shadow dimensions are small and the peristalsis unusually active. A small degree of this aberrancy is indicated by the term anxious, a greater degree by the adjective embarrassed. Both are included in the category of the anxiety complex to distinguish them from the classification of the distress complex outlined in the foregoing paragraph.

Any stomach may on occasion show a flocculent condition of the contained barium. We have noted the appearance of this phenomenon in conditions of temporary agitation. This is much more likely to be associated with the anxiety complex than with the distress complex.

It is our purpose in succeeding communications to develop the themes here indicated in broad outline. On the one hand we intend to present our evidence for the existence of disturbing emotional factors, a subject of great importance for psychological study inasmuch as the indicator is not only beyond the power of voluntary control but in addition, is quite incapable of being dragged up over the threshold of consciousness. On the other hand, with the emotional factor controlled and minimized, we plan to present the evidence for definite behavior patterns in gastric response to particular and constant stimuli.

## SUMMARY

In a former article we put forward the thesis that intrusion of impulses of central (psychologic) origin complicates the reaction patterns of untrained alimentary tracts and is in part responsible for the variation in response characteristic of previous investigations on the subject. In this essay we have subjected the above thesis to a quantitative analysis by such methods as are possible at this time.

From fortunate glimpses of the empty stomach and from a study of the phenomena of filling we have noted the extraordinary facility with which the stomach distends laterally, this distension being apparently induced by a sudden outpouring of juice. Further experiments along these lines will be recorded later.

Owing to the relatively slight change in anteroposterior diameter, dimensions of the barium shadow give a fair degree of probable accuracy to estimates of amount of contents. But while the figures hang well together and give intrinsic evidence of reliability in Sophomores, it is quite impossible to bring the Freshmen results into line. This is due to the unstable character of Freshmen reaction patterns influenced by central impulses which are normally at a minimum in Sophomores.

Especially noteworthy in the gastric reaction pattern is the fluctuation in vertical diameter of the Magenblase.

By suitable training the reaction pattern of gastric response can be stabilized and the human stomach thus prepared for experiments comparable to those of the laboratory.

## ABSTRACT

The thesis that intrusion of impulses of central origin complicates the gastric reaction patterns of untrained subjects is subjected to a quantitative analysis. It is shown that by training the subjects, gastric reaction patterns can be stabilized and the human stomach prepared for experiments of an exact nature comparable to those of the laboratory.

# LABORATORY METHODS

## RESPIRATORY CABINET FOR LABORATORY ANIMALS\*

By J J R MACLEOD ASSISTED BY MISS N R HEARN  
AND F L ROBINSON TORONTO

### RESPIRATORY CABINET

**G**ENERAL Directions.—During the past few years numerous observations have been made in this laboratory<sup>1</sup> on the respiratory exchange of laboratory animals by means of an apparatus devised according to the principles described by Benedict and Tompkins in 1916. This apparatus has been constructed of suitable size for dogs weighing up to 14 kg. It is also perfectly accurate for small animals. It consists in general of an air tight metal box, with a movable lid, ventilated by an air current which passes in, through tubing, at the top of the box and is removed from the bottom the current being maintained by a rotary blower inserted in the outgoing tubing. The air then enters a system of absorbing bottles for the removal of water and CO<sub>2</sub>, and after being again moistened it is returned to the box. On the lid is a Krogh spirometer the movements of which are inscribed on a smoked surface. As the volume of air in the system becomes less owing to the absorption of CO<sub>2</sub>, the descent of the spirometer makes contact in an electric circuit which thereby operates an electromagnet so that a clamp, which is compressing a thin rubber tube through which oxygen is passing is opened and O<sub>2</sub> flows from a tank at uniform low pressure through a Bohr meter, into the inlet tube of the apparatus.

The CO<sub>2</sub> expired by the animal is measured by weighing the absorption bottles, and the oxygen by the volume recorded on the meter. Correction is also made for variations in volume of the air in the chamber due to changes in temperature and barometric pressure. For this purpose several thermometers, reading to 0.05° C., are inserted in the chamber one of which extends to its bottom and three are near the top. Two of the latter are close together, one of them being a wet bulb thermometer, and a small fan is inserted through the walls of the chamber so as to direct a constant air current onto it. This serves as a psychrometer for measurement of the aqueous tension.

For the purpose of testing the accuracy of the apparatus, a measured quantity of alcohol is burned in a cylinder inserted in the system of tubing.

The general plan of the apparatus is shown in Fig 1† and a photograph of it is also shown in Fig 3.

<sup>\*</sup>From the Physiological Laboratory, University of Toronto, Canada.

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<sup>†</sup>The balance used for weighing the absorption bottles was supplied by Aug. Sauter, Uebingen, Württemberg, Germany. (Precision Balance No. 7, 10 kg.)

## DETAILED DESCRIPTION

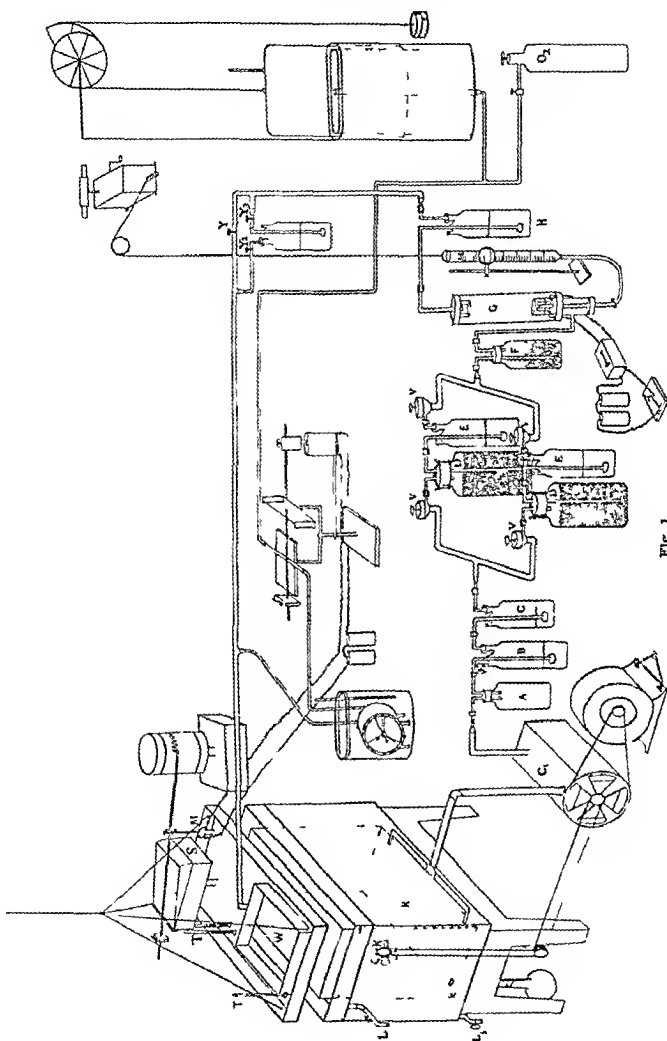
1 *Respiratory Chamber (Fig 1)*—This is made of galvanized iron, 22 gauge. It is double walled, the dimensions of the outer casing being 47 by 89 by 52 cm and those of the inner one, 38 by 80 by 45 cm. The space between the two walls is filled with water. At a distance of 5 cm from the top, the space between the two walls is bridged across so as to form a water trough into which the lid of the box fits. The water in the space below is kept stirred up by means of an air current delivered through a perforated tube at the lower end of the box. The lid, which can be raised by a pulley, measures 43 by 86 cm and is provided with a flange 5 cm deep which fits into the water trough. It also has a glass window, *W*, 30 by 30 cm, and smaller openings for the insertion of the thermometers, *T*. One of these, at a corner of the lid, is for a long-stemmed thermometer which reaches to the lower end of the box (not shown in figure). The stem of this thermometer is protected by a metal tube, slotted along one side, the slot being covered by stout wire gauze. Two other thermometers are placed about the center of the box and their bulbs project just below the lid. One of these is kept moist by means of a wick which dips into a small bottle filled with water, the pair therefore serving for the measurement of humidity. On one side of the box is an opening through which, with suitable packing boxes, passes the shaft of a small fan, *K*, driven by a belt coming from the blower-motor. Another thermometer is placed at the corner of the lid farthest from the long thermometer.

The Krogh spirometer, *S*, is directly mounted on the lid and is connected with the chamber by a  $\frac{3}{4}$  inch tube. This spirometer has a capacity of 1800 cc and its lid, which is carefully counterpoised, is connected with a long lever which inscribes the movements on a travelling surface, and has connected with it at right angles an arm which passes down to a mercury cup, to complete the electric circuit referred to above. There is sufficient give in the walls of the box so that any movements of the animal are distinctly recorded by the spirometer. The outer case of the spirometer is made of copper and the lid of very thin zinc (16 gauge), the metal surfaces being carefully covered with shellac.

We have found it of advantage to have the lid heat-insulated, by covering it with a deep layer of broken cork held in position by flanges along the edges. For raising and lowering the lid it is necessary to empty the water out of the trough, and this is done by means of a  $\frac{3}{4}$  inch pipe connected at one corner *L*. The water in the water jacket may similarly be evacuated by means of a  $\frac{3}{4}$  inch pipe at the lower corner *L'*. Finally two narrow tubes are inserted, one through the lower part of the box and the other through the lid for the purpose of removing samples of air for analysis.

Resting on the floor of the chamber is a flanged tray, which slopes slightly toward one end where there is a pipe connected outside, by means of rubber tubing, to a flask for the collection of urine. An inner tray of wire mesh prevents the animal from resting directly on the sloping tray. These trays are easily taken out for washing, etc.

2 *Absorbing System*—The air is taken from the lower end of the box by a  $\frac{3}{4}$  inch pipe which branches inside the water jacket so that its openings are





at the lower corners of the box. Immediately after leaving the box the pipe is joined by a brass coupling with the negative side of a Crowell blower, *Cr* (Crowell Mfg Co., Brooklyn, N. Y. No. OD rotary compressor), which is placed in an iron box filled with oil. A suitable ventilation, about 15 liters per minute, is obtained when the motor connected with the blower is rotating at a speed of 110 revolutions per minute.

The positive side of the blower is connected with a wide-mouthed bottle, *A*, of about 1 liter capacity, to serve as a trap preventing any sulphuric acid being accidentally sucked back into the pump. The pipe leading from this bottle is coupled with a large Williams bottle, *B* (of 2 liters capacity), containing 750 cc concentrated sulphuric acid. In order to make the union between glass and metal air-tight, the tubes of the Williams bottles are fitted with brass female couplings of standard,  $\frac{3}{4}$  inch size, by placing a piece of rubber tubing over the glass tube and then turning the coupling so that it fits snugly on this tube. If any leakage should occur this may be stopped by Chatterton's cement. The third tube of this Williams bottle, *B*, is provided with a glass stopcock which is used for adjusting the pressure of the system at the start of an observation. A second Williams bottle, *C* (of 1 liter capacity), containing 500 cc sulphuric acid, follows and its outlet tube is connected, through a coupling, and a piece of wide rubber tubing with the T-piece which carries the valves, *V*, the construction of which will be described in detail below.

After passing through either valve the air is led to the  $\text{CO}_2$ -absorbing bottle, *D*, which can be quickly detached by unloosening the couplings connecting it with the valve and the Williams bottle containing sulphuric acid, *E*, which next follows. This connects with a second pair of valves and a T-piece, the central tube of which is coupled to a pipe leading to the bottom of a wide-mouthed bottle, *F*, which is filled with cotton to collect any sulphuric acid spray which may come over. The outlet tube of this bottle enters the lower end of a glass cylinder, *G*, of 7 cm diameter and  $28\frac{1}{2}$  cm long for burning alcohol, the upper end being connected, through suitable couplings and a short piece of rubber tubing, with a large-sized Williams bottle, *H*, which contains water for the purpose of moistening the air before it returns to the chamber which it does through a pipe opening in the center of the lid. On this pipe is a side tube which delivers oxygen into the system through a Bohr meter at a rate regulated by the electromagnetic valve. In the drawing the tubing near the absorption bottles is represented as being of smaller diameter than elsewhere. This is not actually the case.

**Valves.** Since some trouble was experienced in obtaining, on the market, valves that were satisfactorily air-proof, special ones were designed, as shown roughly in Fig. 2. Each consists of a cylindrical portion, 2 cm high and 6 cm in diameter, and a funnel-shaped portion narrowing down to an opening of 2 cm through a distance of 4 cm and provided with a side-tube of standard ( $\frac{3}{4}$  inch) size. Between the cylindrical and funnel-shaped portions is an internal ledge 5 mm wide and 1 mm in thickness. This part of the valve is cast in brass. The cylindrical portion is threaded on the inside (32 to the inch) to take the flange of a brass cover, and through the center of the cover passes

a spindle, threaded 8 to the inch. This terminates in a handle above and is rounded into a small ball below. A diaphragm, 6 cm in diameter, cut out of rubber, 5 mm thick rests on the ledge on to which it is pressed down by the flange of the lid, a cast washer 5 mm thick being interposed between. Through the center of the diaphragm passes a brass rod ( $\frac{3}{16}$  inch) ending above in a socket which fits the ball shaped end of the spindle. This rod is threaded for a nut which is screwed up against the rubber diaphragm and socket. Its lower end passes through a rubber stopper which is held in position on the rod by two nuts and is shaped so as to close the lower opening. The valves are connected up as shown in the diagram of Fig 1. These valves have given very satisfactory service and are completely leak proof, provided the rubber diaphragm and stoppers are replaced about once every month during daily use of the apparatus.

**The CO<sub>2</sub> absorbing bottles.** Wide mouthed glass bottles of 1 liter capacity furnished with a brass cover carrying the inlet and outlet tubes have proved much more satisfactory than those usually employed. A circular cast brass

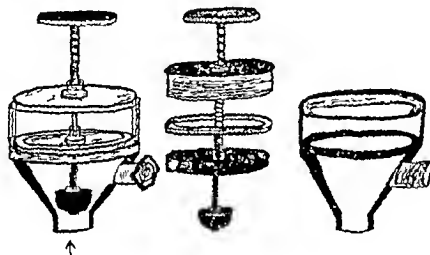


Fig. 2

plate (of the same diameter as the mouth of the bottle) has two brass tubes of standard size passing through and brazed on to it. To one of these is soldered a lead tube long enough to extend to the bottom of the bottle. The brass lid is clamped down, in the manner shown in Fig 1 to a brass collar which is cemented on to the neck of the bottle by means of aquarium cement. Wilson soda lime 4-8 mesh (supplied by Warren Collins Boston, Mass.) is used to absorb the CO. One kilo of this absorbs from 60 to 70 gm of CO and when there is doubt as to whether absorption is proceeding efficiently the air current is deflected through a Williams flask containing barium water by suitable adjustment of the clamps  $Y_1$ ,  $Y_2$ , and  $Y_3$  (Fig 1).

Further details of the apparatus are sufficiently given in the sketch plan and photograph (Fig 3).

*The calculation of the results* is carried out exactly as described by Benedict and his coworkers. In the observations made by us the CO bottles have been weighed at intervals of an hour when also the meter and the thermometers were read. The total duration of each observation was usually eight hours. At periods samples of air were removed from the chamber for analysis in a Haldane gas burette and if any considerable deviation was found

in the percentage of  $\text{CO}_2$  the accuracy of the respiratory observation was placed in doubt. Experience soon showed, however, that little error could be incurred on account of an unequal rate of absorption of  $\text{CO}_2$ , especially when the animal was quiet, so that this check came latterly to be little used. Chance of error on account of the omission was guarded against, by placing the animal in the chamber with absorption apparatus in position for at least one-half

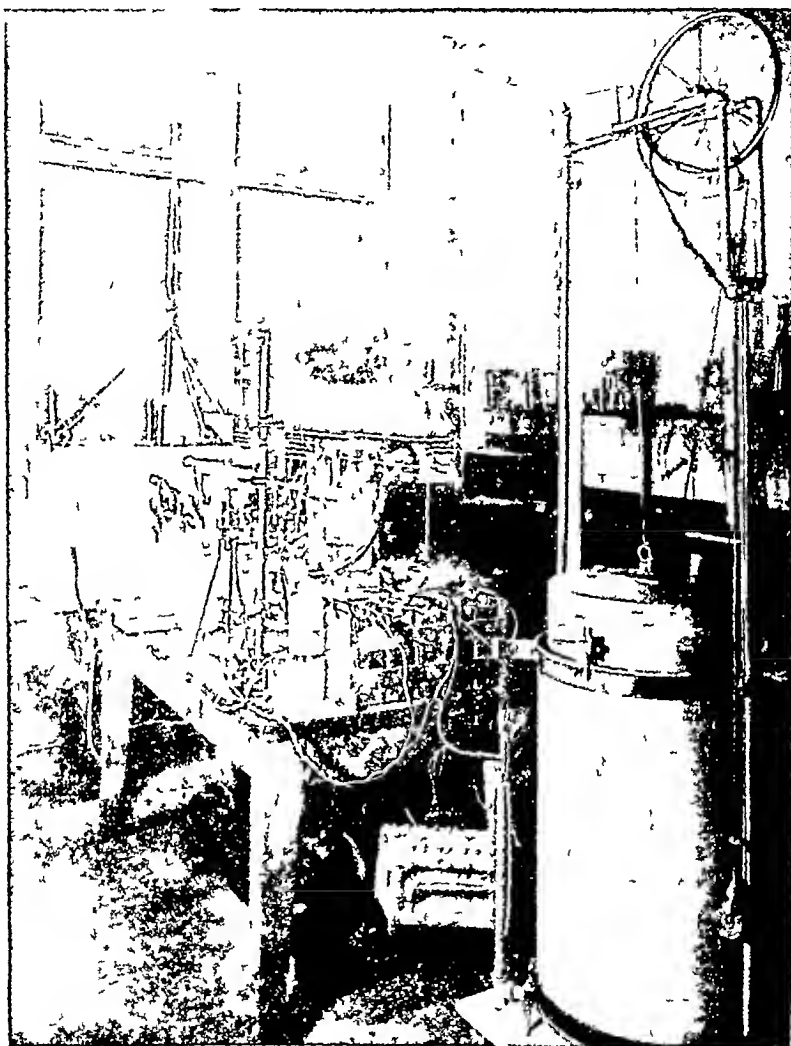


Fig 3

hour before actually using weighed bottles, or reading the meter. During this preliminary period, also, the volume of the air in the system was allowed to become constant, its expansion, due to the heat given off by the animal, being corrected by opening the escape valve on *B*. Most of the animals after a short experience in the chamber became quiet and indeed often slept throughout the observation. Their movements were recorded on the smoked drum, and if these proved to be considerable the result was put in question since, under

such conditions, CO might be produced at a rate in excess of that at which it was being absorbed by the soda lime. However, since the observation was continued for several hours and the animal usually was quietest toward the end, any error due to this cause was eliminated in the average result for the day.

**Accuracy of apparatus** This was tested in two ways (1) By seeing whether the volume of air remained unchanged without having an animal in the box, but with the pump running. This was usually tested for several hourly periods and was found to be a reliable method for detecting leaks. (2) By burning a known volume of alcohol in the glass cylinder placed in series in the absorption system as shown in Fig 1. The burner for the alcohol and the manner of feeding it with alcohol from a burette are also shown, and it may be said that after the correct rate for raising the feeding burette has been determined by adjusting the speed of the clock by which the thread supporting it is wound (see Fig 1) there is no technical difficulty in applying this check. At first we attempted to place the alcohol burner in the respiratory cabinet but the results were unsatisfactory because of the sudden changes in volume of air occurring not only when the flame was lighted but during its burning. This also was usually more or less irregular, probably owing to variability in the oxygen supply in the immediate neighborhood of the flame. This irregularity meant that combustion was not always complete and a smell of unburned alcohol could often be detected when the lid was removed from the chamber. Moreover while in the chamber the alcohol flame often went out, which seldom occurs when the cylinder is used. If it does go out, the flame is readily started again by the sparking of a high tension electric current. Another source of error incurred with the flame in the chamber is owing to drying of the air. This is avoided by the cylinder method because of the moistener, *H*.

Table I gives the results of some alcohol tests run during the spring of the present year and they show satisfactory agreement with regard to both O<sub>2</sub> consumption and R Q.

Taking all observations together there is an excess of 71 c.c., or 0.26 per cent of CO<sub>2</sub> and an excess of 304 c.c. or 0.76 per cent of O while the average R Q is 0.664 the theoretical being 0.667. Since the error in both CO and O

TABLE I

DATE	DURATION OF PERIOD	ALCOHOL BURNED	CO PRODUCED		O USED		R.Q.
			FOUND	THEOR.	FOUND	THEOR.	
1928		93.44% at 20° C					THEOR. 0.66
	hr min sec	cc	cc	cc	cc	cc	
Mar 1	1 54 0	3 c.c.	2274	2252	3397	3370	0.669
" 12	1 35 0	6 c.c.	4490	4504	6711	6740	0.669
" 15	0 40 0	3 c.c.	2124	2162	3181	3244	0.668
" 27	1 01 30	3 c.c.	2160	2175	3273	3264	0.660
" 27	0 59 30	3 c.c.	2137	2198	3243	3798	0.69
Apr 3	0 58 30	3 c.c.	2059	2193	3394	3291	0.666
May 11	1 07 0	3 c.c.	2193	2194	3334	391	0.69
" 11	1 19 0	3 c.c.	2198	2194	3333	3291	0.69
" 16	0 40 0	3 c.c.	2173	2198	326	3798	0.667
" 30	1 02 0	3 c.c.	2264	2198	3380	3293	0.60
" 30	1 10 0	3 c.c.	2305	2198	3485	398	0.66
Total		36 c.c.	26537	26466	39957	39683	Av. 0.664

measurements is sometimes *plus* and sometimes *minus* in the different experiments, it must be incurred, not because of any slight leak in the apparatus, but owing to inaccuracy in weighing the CO<sub>2</sub> absorbers, and probably also because we have to depend on wet- and dry-bulb thermometers for determination of the water vapor. In any case, the error is of little consequence in the type of observation for which the apparatus has been used. Besides these special tests for accuracy any error due to leakage, or other cause, could usually be detected in the course of a series of observations by watching the behavior of the R Q. In depancreatized dogs this is remarkably constant for at least the first week after withdrawal of insulin and food, so that any fault in the technique was usually revealed by an abnormal quotient during the first hour of the observation.

## REFERENCES

- 1 Macleod, J. J. R. Carbohydrate Metabolism and Insulin (Monographs on Physiology), 1926, Longmans Green & Co.  
Campbell, W. R., and Markowitz, J. J. Clin. Invest. 4: 37, 1927, Am. J. Physiol. 80: 548 and 561, 1927.  
Chaikoff, I. L. J. Biol. Chem. 74: 203, 1927.  
Chaikoff, I. L., and Macleod, J. J. R. Ibid. 83: 725, 1927.  
Chaikoff, I. L., and Weber, J. J. Ibid. 1928.  
Markowitz, J., and Macleod, J. J. R. Trans. Assn. Am. Physicians, 41: 147, 1926.  
Markowitz, J. Am. J. Physiol. 83: 698, 1928.
- 2 Benedict, F. G., and Tompkins, E. H. Boston Med. & Surg. Jour. 174: 1916.

## STABILITY OF DESICCATED BEEF HEART MUSCLE POWDER FOR PREPARATION OF ANTIGENS FOR COMPLEMENT-FIXATION TESTS IN SYPHILIS, WITH SPECIAL REFERENCE TO THE KOLMER METHOD\*

BY ROBERT A. KILDUFFE,† A. M., M. D., AND BETTY SHIFMAN, ATLANTIC CITY, N. J.

ONE of us (R. A. K.) has previously reported<sup>1</sup> that commercially prepared desiccated beef heart powder‡ was quite stable and suitable after the expiration of eleven months for the preparation of antigens satisfactory for the conduct of complement-fixation tests in syphilis by Kolmer's method.

Recently, through the courtesy of Mr. H. G. Dunham of the Digestive Ferments Co., we have been able to test beef heart powders varying in age from sixteen to sixty-four months with the results shown below.

Both the old and new titrations were carried out in exact accordance with the method described by Kolmer.<sup>2</sup>

It is of interest to note that the powders were kept at room temperature and without special precautions in cork-stoppered bottles.

\*From the Laboratories of the Atlantic City Hospital.

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†Director Laboratories Atlantic City Hospital.

‡Digestive Ferments Co.

NO	DATE POWDER MADE	ORIGINAL TITER	NEW TITER	AGE OF POWDER WHEN NEW ANTIGEN MADE
11631	5/ 8/23	Hem not in 1 4 Antic 1 6 Antig 1 2000	Hem not in 1 4 Antic 1 20 Antig 1 4000	64 months
12145	11/21/23	Hem not in 1 4 Antic 1 6 Antig 1 2400	Hem not in 1 4 Antic 1 20 Antig 1 3200	59 months
12692	6/23/24	Hem not in 1 4 Antic 1 6 Antig 1 3200	Hem not in 1 4 Antic 1 12 Antig 1 1200	52 months
13564	2/16/25	Hem not in 1 4 Antic 1 6 Antig 1 4000	Hem 1 6 Antic 1 32 Antig 1 4000	44 months
14375	9/ 4/25	Hem not in 1 4 Antic 1 16 Antig 1 4000	Hem 1 4 Antic 1 32 Antig 1 4000	37 months
17019	6/24/27	Hem not in 1 4 Antic 1 8 Antig 1 3200	Hem not in 1 4 Antic 1 16 Antig 1 4000	16 months

The conclusions formed from the previous investigation may be again repeated but with added emphasis namely

1 Under ordinary conditions of storage desiccated beef heart powder is quite stable satisfactory antigens having been prepared from it after five years and four months

2 The only change encountered was a slight increase in the anticomplementary value in no case sufficient to interfere with the antigenic efficiency of the extract

3 Beef heart muscle powder may be prepared commercially and stored in large quantities for at least five years without fear of undue deterioration

#### REFERENCES

- 1 Kilduffe R A Stability of Desiccated Beef Heart Muscle Powder for Preparation of Antigens for Conduct of Complement Fixation Tests in Syphilis Arch Dermat & Syph 10 734 1924
- 2 Kolmer J A A New Complement Fixation Test for Syphilis Based Upon the Results of Studies in the Standardization of Technic Am J Syph 6 1 1922

# METABOLISM MASK FOR DOGS\*

BY REBEKAH GIBBONS, OMAHA, NEBRASKA

A MASK suitable for connecting dogs to any common type of metabolism apparatus has been devised. It has the following advantages:

- 1 The animal's eyes need not be covered during the tests. The operator may thus more easily assure himself that the animal is awake.
- 2 The animal is only very slightly disturbed in adjusting the mask.
- 3 The rebreathing volume is very small.
- 4 The pressure necessary to assure an air-tight connection is borne by tissues supported by jaw and cheek bones. Circulation and respiration are not interfered with. The greater comfort from this mask may be determined subjectively by encircling one's neck firmly but lightly with the hands and comparing the mild discomfort resulting, with the lack of such discomfort when considerable pressure is exerted for the width of a single finger to the bony parts of the nose and the cheek and jaw bones.
- 5 There is no chance of the mask tearing at an inconvenient moment as is the case when the animal's mouth parts are forced into a small hole cut in sheet rubber.
- 6 The animal's mouth and lips may be comfortably arranged before the mask is tightened. Creases in the skin, even with dogs having redundant lips, may be more easily eliminated.
- 7 The flexibility of the mask allows a slight pulsation during both inspiration and expiration, thereby buffering pressure changes in the mask and further helping to obviate leaks.

The flexible mask constructed entirely of rubber is shown in the illustration.

## DESCRIPTION OF THE MASK

The mask is cylindrical in shape and carries in one of the bases a piece of rubber tubing of suitable size and length by which it may be connected to the respiration apparatus. After it has been comfortably adjusted with the animal's lips pushed far forward and the open portion of the mask pulled well up toward the eyes, it is made air-tight by wrapping a strong rubber band approximately one-half inch wide twice around the mask and fastening it with a hemostat. On the first turn the band is stretched as much as is possible without causing puckers in the rubber of the mask. On the second turn the band is stretched as tightly as is consistent with the comfort of the animal. Care should be exercised that the animal's lips are in front of the band. If the mask is correctly adjusted well up toward the eyes, the considerable pressure of the band is almost entirely borne by tissues well supported by cheek and jaw bones. Ease of respiration is not interfered with nor is the circulation hampered, and the animals submit without muscle tension to considerable stretching of the rubber band. As originally conceived, the mask was cone shaped to obviate

\*From the University of Nebraska.

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rebreathing in so far as possible but the pressure on the soft tissues toward the nostril was great enough to cause discomfort. This is not the case with the cylindrical mask which when in place assumes the shape of a broadly truncated cone.

#### THE USE OF THE MASK

With moderate tension of the band an air tight connection can be secured on dogs with short soft hair without showing leaks. For dogs with wiry hair such as Airedales, the band must be considerably tighter but satisfactory tests may be easily secured. The omission of shaving saves considerable time as well as the annoyance of an occasional skin wound or other abrasion which sometimes interferes with a satisfactory determination at a time when it is desirable to test the animal.

#### TESTING THE MASK

The tracings which have been reproduced were made from unshaved dogs using a Benedict portable respiration apparatus. A clockwork kymograph was used for the graphic records. The slope of the respiratory tracing therefore

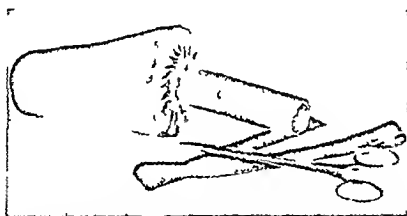


Fig 1—Cylindrical rubber mask for attaching dogs to metabolism apparatus. Also the heavy rubber band and hemostat used to produce an air tight connection.

becomes a measure of the intensity of the metabolism. The arrow on the tracing indicates the removal of a 40 gm weight from the spirometer bell. Tracing I shows a typical satisfactory test. The fall of the bell for the 'weighted' and 'unweighted' portions of the test period was the same. Tracing II shows the effect of a small leak resulting from insufficient tightening of the rubber band. The slope of the line indicating the fall of the spirometer bell is sharper for the 'weighted' than the 'unweighted' part of the test period.

#### DIRECTIONS FOR MAKING THE MASK

To make a mask such as the one here described a rectangular section is cut from sheet rubber. One dimension equals 7 mm less than the circumference of the dog's muzzle just below the eyes. This provides for a 7 mm lap making the finished mask smaller in circumference than the part of the dog's face to which it is to be applied. This allows enough stretch so that it will fit snugly and hold the angle of the lips well forward, but at the same time permits comfortable adjustment and easy breathing. The other dimension equals the distance from the position which the top of the mask will occupy when in place on the dog, and the tip of the animal's nose plus 30 mm. This provides for



20 mm between the end of the dog's nose and the outlet tube in the finished mask and a 10 mm allowance to turn up and cement onto the base. A circular section with a circumference equal to the long dimension of the rectangular piece less the lap is also cut for the base.

The ends of the rectangular piece should be lapped and vulcanized. Care should be exercised that the cushion rubber receives sufficient pressure so that there is no ridge at the seam. A better turn can be made over the base if the cushion rubber is cut an inch shorter than the mask rubber at this edge. There is no strain on it and the cement will hold without reinforcement.

The lower edge of the resulting tube is notched for turning onto the base. The V-shaped pieces removed being 10 mm deep and a trifle wider than those

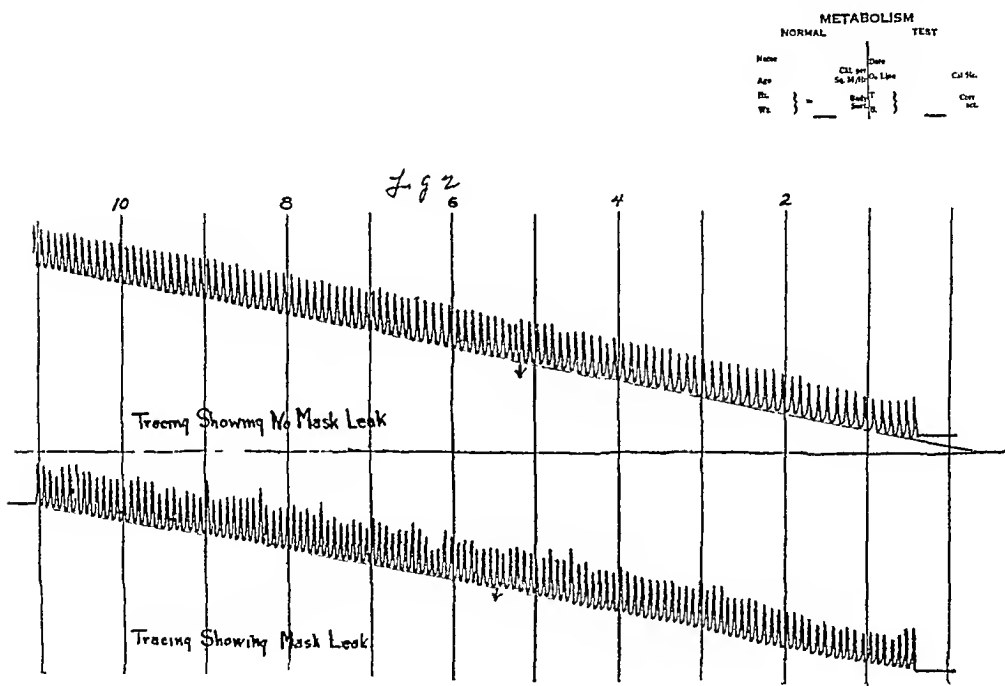


Fig 2

which are left. The V's which make up the saw-toothed edge should not be over 7 mm wide if a smooth turn is to be made.

The mask is most easily put together if a bottle or can of the same diameter as that of the mask can be found. The notched tubular part is slipped over the bottle or can to within 3 mm of the notched edge and the cement applied. Even pressure can now easily be exerted in cementing it onto the circular base. It may either be vulcanized or cold cemented. This part of the mask is not submitted to stretching so that vulcanizing is not necessary and is probably undesirable, for this part is awkward to vulcanize and occasionally an over-cured spot results.

After the completed cylinder has been tested for air-tightness a small circular hole about 5 or 6 mm in diameter is cut in the center of the base, and the large tubing by which the mask is attached to the apparatus is introduced and cemented into place.

## THE DETECTION OF BLOOD BY MEANS OF BENZIDINE DIHYDROCHLORIDE\*

BY S L LEIBOFF, M A, NEW YORK CITY

**A**MONG the various tests for the detection of blood in biologic materials, the guaiac and the benzidine tests are the most widely used. The guaiac is less sensitive and less reliable than the benzidine test. This is due chiefly to the fact that gum guaiac is an impure substance of indefinite composition. White,<sup>1</sup> who has studied a large number of cases with gastric ulcer, found that guaiac often failed to detect blood in feces while benzidine detected blood in all these cases. However, the test with benzidine must be carefully adjusted as benzidine, particularly when applied to feces, may be too sensitive and give false positive results. Meat, fish, iodides, and iron salts give positive results. After eating meat a positive test may be obtained with benzidine for three days after the meal.

Lyle and Curtman<sup>2</sup> prefer guaiacomic acid for the detection of blood in feces, since it is not as sensitive as benzidine and is more sensitive than guaiac. They describe a method for the preparation of this reagent.

Ruttan and Hardisty<sup>3</sup> found that acetic acid solutions of benzidine rapidly deteriorate in sensitivity toward blood, thus necessitating the use of freshly prepared solutions. They recommend the use of ortho toluidin, which is benzidine containing two methyl groups.

Lyle, Curtman and Marshall<sup>4</sup> found a number of factors to influence the accuracy of the benzidine test. They found that alcoholic solutions were not reliable, as positive tests were obtained with controls containing no blood. The ratio of the reagents used in the test must be properly adjusted. This is particularly true of the acetic acid and the hydrogen peroxide. The acetic acid serves merely to keep the benzidine in solution. An excess of acetic acid diminishes the delicacy of the test by increasing the speed with which the color fades. The purpose of the hydrogen peroxide is to supply oxygen. When too little hydrogen peroxide is used very little color develops while an excess destroys the catalytic power of the blood and reacts with the benzidine itself.

The purpose of this paper is to eliminate the objectionable reagents, to prepare a stable benzidine solution and to standardize the technique as to the proper ratios of the reagents used.

Benzidine combines easily with a great many substances and produces many highly colored compounds. It is easily oxidized to form colored substances, and upon this fact is based its use for the detection of blood, the peroxidases in the blood break up the  $H_2O_2$  into water and liberate free oxygen which in turn oxidizes the benzidine producing the blue color.

From the Biochemical Laboratory of Lebanon Hospital, New York.  
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the higher the concentration the more color developed This is shown in Table II

The blue color fades rapidly depending upon the amount of hydrogen peroxide, the more hydrogen peroxide used the quicker the color fades Thus the tubes in Table I, where only a half cc of hydrogen peroxide was used began to fade within ten minutes, while those in Table II where 2 cc of peroxide were used, faded within three minutes When a great excess of hydrogen peroxide was added no color developed but a white precipitate formed When the color fades it is not destroyed but is transformed into a cherry red color which is quite stable

A few other oxidizing agents were tried in place of the hydrogen peroxide, such as benzoyl peroxide, barium peroxide, and sodium persulphate, but none of them proved as good as the hydrogen peroxide

By using the proportions of the reagents described in the method, a good color is obtained in dilutions of 1 to 500,000

Meat extracts, peptone, pepsin, trypsin, and amylase gave no color with the reagents used Iron salts and iodides do interfere with the test With iron salts a deep green color is obtained With iodides a black green precipitate is obtained, and the solution is colored a deep yellow

TABLE I

TUBE	BLOOD (1 10,000)	BENZIDINE	H <sub>2</sub> O (3 PER CENT)	*COLOR
1	5 cc	01 cc	05 cc	2
2	"	02 "	"	3
3	"	03 "	"	4
4	"	04 "	"	5
5	"	05 "	"	4
6	"	07 "	"	3
7	"	10 "	"	2
8	"	20 "	"	1
9	"	50 "	"	?

TABLE II

TUBE	BLOOD (1 10,000)	BENZIDINE	H <sub>2</sub> O (3 PER CENT)	*COLOR
1	5 cc	01 cc	2 cc	1
2	"	02 "	"	2
3	"	03 "	"	3
4	"	04 "	"	4
5	"	05 "	"	5
6	"	07 "	"	8
7	"	10 "	"	10
8	"	20 "	"	15
9	"	50 "	"	30

\*The intensity of the color is expressed numerically. Number 1 expresses the slightest amount of color observed. The figures are approximate

In the determination of blood in feces, the tube must be cooled before the reagents are added as otherwise the blue color will go over into the cherry red color immediately

It is very important to add the hydrogen peroxide last or no color will be produced

The test was applied to a large number of urines and feces and proved very efficacious. No positive tests were given when no blood was present, as determined by other methods.

## SUMMARY

1 A simple method is described for detecting blood in urine and in feces by the use of benzidine dihydrochloride

2 A simple method is described for the preparation of pure benzidine dihydrochloride from commercial crude benzidine base

## REFERENCES

- 1 White F W Boston Med & Surg J 164 876, 1911
- 2 Lyle, W G and Curtman L J J Biol Chem 33 1, 1918
- 3 Ruttan and Hardisty Biochem Bull 2 225 1912
- 4 Lyle, W G, Curtman L J and Marshall J Biol Chem 19 415 1914

## A SIMPLE METHOD FOR STAINING SPIROCHETES\*

By EMIL WEISS M D CHICAGO, ILL

THE review of the literature shows a considerable number of methods recommended for the staining of the spirochetes. Only few of them are actually in general use due to the fact that most of these methods do not fulfill the fundamental requirements for staining procedures, which are (1) The applied stain must be effective (2) the stain must keep well, (3) the procedure must be as simple as possible. The present methods which are used for the demonstration of spirochetes can be classified in four groups (1) Stains otherwise used for the demonstration of blood cells, (2) methods resembling those used for the staining of flagella (3) methods with negative pictures (background stained spirochetes unstained) (4) vital staining.

In using blood stains the spirochetes take the eosin. The affinity of spirochetes for acid dyes is of considerable importance for the interpretation of their biologic status. Bacteria preferably take basic dyes while the animal cells show more affinity for the acid dyes. The nuclei of animal cells take basic dyes. The staining reactions of spirochetes with blood stains indicate a closer relationship to the animal kingdom than to the plant kingdom. The different blood stains demonstrate distinctly the morphology of spirochetes in relation to tissue elements particularly blood cells. The procedures which resemble the flagella methods use first a mordant, then a basic dye or a metallic salt or a combination of both. The use of metallic salts has one great disadvantage that the formation of sediment cannot be avoided and may cause in some instances doubt concerning the interpretation. The negative methods are undoubtedly inferior to the above methods and may easily cause oversight of the possibly present spirochetes.

The author thought it worth while to compare the different flagella methods as to their usefulness for the demonstration of spirochetes. He found that most of the flagella methods are applicable for the demonstration of spiro

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chetes The chief difficulty lies in the fact that the various mordants used by the flagella methods do not keep, and must be renewed at short intervals or filtered before use Another difficulty lies in the complicated technic of some flagella methods, which require considerable experience to obtain satisfactory results The author tried to meet these and some other objections in a recent paper on staining of flagella<sup>3</sup> Further studies also showed that this method is equally useful for the staining of spirochetes

#### THE PRINCIPLES OF THE METHOD

*Preparing of Films*—The fresh material is carefully placed on a slide and diluted in a drop of 5 per cent glacial acetic acid The slide is inverted and placed over a hollow ground slide in a manner as usually practiced for the preparation of a hanging drop A too fast drying of the drop can be avoided by the use of vaseline The slides with the drop are placed in the incubator for fifteen minutes The vaseline is then removed with xylol and alcohol The drop is carefully spread over the slide and dried in air For routine purposes the material is immediately smeared, allowed to dry and then the mordant is applied If desired the filming of spirochetes can be preceded by vital staining (Mandelbaum, Merowsky<sup>1</sup>) The same drop can be utilized for the subsequent application of our method

*The Mordant*—A comparative study of various mordants shows that tannic acid is the most important constituent The other chemicals are of secondary importance The deterioration of the majority of mordants is caused by the fact that tannic acid does not keep well in watery solutions The great solubility of tannic acid in alcohol was utilized to develop a stable mordant One hundred grams of tannic acid were dissolved in 100 cc of 95 per cent alcohol (Sol A) and before use mixed with two parts of undiluted formalin containing 75 per cent glacial acetic acid (Sol B) This mixture contains about 20 per cent tannic acid Both solutions are stable and do not require repeated filtering This mordant resembles somewhat Fontana's mordant, which is not stable In Fontana's method the fixative and the mordant are used separately In our method the fixative and the mordant are contained in stable solutions and their application occurs simultaneously The essential constituents (tannic acid, glacial acetic acid, and formalin) are contained in our mordant in a concentration 4 to 5 times higher than in Fontana's method

*The Use of the Mordant*—The air-dried smears or films are covered with the mordant and steamed slowly for two to five minutes The removal of the excess of the mordant is most satisfactorily obtained with running warm water

*The Use of Basic and Acid Dyes*—The bacterial flagella and spirochetes show similarity in staining reactions with basic dyes The bacterial bodies take the basic dyes more heavily than the bacterial flagella or spirochetes This difficulty can be partially overcome by using saturated solutions of basic dyes, by longer staining, or by application of heat while staining (steaming) Instead of basic anilin dyes metallic salts can be successfully used The affinity of flagella for acid dyes has been demonstrated in a previous paper<sup>3</sup> An intensive staining of the spirochetes can be accomplished by staining first with a saturated solution of a basic dye, and then with an acid dye of the same color

(crystal or gentian violet and acid violet basic and acid fuchsin, brilliant green and acid green, methylene blue and acid blue etc.)

*Contrastive Staining With Basic and Acid Dyes*—This method also devises a procedure for a contrastive staining of flagella by the use of basic and acid dyes. This procedure can also be successfully applied for a contrastive staining of spirochetes. In this instance the bacterial bodies and the nuclei of tissue cells take the basic dyes, the spirochetes, the bacterial flagella, the bodies of degenerated bacteria and the cytoplasm of tissue cells take the acid dyes. Such a procedure is very effective in instances where the smears contain besides the spirochetes a variety of bacteria and tissue elements (for instance, Vincent's angina). The following combinations of contrasting basic and acid dyes are recommended for use: saturated aqueous or alcoholic solutions of crystal or gentian violet and acid green, safranine or fuchsin with acid green, brilliant green with acid violet or acid fuchsin.

*Staining Procedure*—The slides which were thoroughly washed with water after application of the mordant, are covered for two to five minutes with the saturated aqueous or alcoholic solution of the respective basic dye, washed off with warm or cold water and then covered for eight to ten minutes with the saturated solution of the acid dye which is dissolved in 30 per cent alcohol. The slides are then washed with water and allowed to dry in the air without application of heat.

#### DISCUSSION

A method has been described for the staining of spirochetes, which was already found useful for the demonstration of flagella. The spirochetes appear as large as with any of the previous methods, which use mordants. Several dyes can be used with the same results. By the use of basic and acid dyes a contrastive staining between spirochetes and bacteria can be obtained. The special advantages of this method are: (1) The simplicity of the technique, (2) the mordant keeps indefinitely, (3) no sediment is formed, (4) choice between several dyes, and (5) contrastive staining.

#### SUMMARY

A new method for staining of spirochetes has been devised with the following procedure. The material supposedly containing spirochetes is placed on a slide in a drop of 5 per cent glacial acetic acid. The slide is inverted over a hollow ground slide and placed in the incubator for fifteen minutes. The drop is then spread and allowed to dry in air. The slide is covered with the mordant and steamed for two to five minutes. The slide is then washed with warm water and covered with a saturated solution of one of the recommended basic dyes for two to five minutes, the slide is then washed with water and dried in the air. If a contrastive staining is desired, the slide is then covered with a 10 per cent solution of a contrasting acid dye in 30 per cent alcohol for eight to ten minutes, washed with water and dried.

#### REFERENCES

1. Kolle W. and Wassermann, A. (Friedberger u. Reiter) Handb. d. Path. Mikroorg., 1: 370-381, 1912.
2. Ford W. W. Textbook of Bacteriology 1927, p. 76. Saunders Co. Philadelphia.
3. Weiss E. Method of Staining Flagella. J. Infect. Dis. 43: 228, 1908.

# AN AUTOMATIC MICROTOME KNIFE SHARPENER AND METHODS FOR GRINDING AND HONING THE KNIFE SATISFACTORILY\*

BY JOHN I. FANZ, M.D., PHILADELPHIA, PA

HAVING run the gamut of razor blade holding devices, having tried all types of hones (including the better grade Belgians), I resurrected the glass plate grinding method of Chas S Minot. This produced results far superior to those obtained with the stone hone, but the time required to condition the knife extended over a period of many hours, especially when the knife presented the coarser variety of nicks. To move the attrition glass plate by motor at a suitable speed was the first step in the evolution of the sharpener. To select proper abrasives and to develop methods for their use was the second and most annoying search to make the automatic sharpener a success.

A full description of the instrument would be too lengthy for the present article. Figs 1 and 2, however, will give a fair idea of the instrument. The 18-inch ground glass disc is rotated at a speed of 42 revolutions per minute, toward the knife edge in all processes of grinding, honing and polishing. Stropping is accomplished on a paper disc covering the glass plate, the movement during this operation being away from the edge. This is accomplished by swinging the knife holder across the disc into an opposite radius. The oblique draw of hand honing is simulated by a reciprocating device, moving the knife inward and outward in the radius of the moving disc 30 times per minute.

## MATERIALS USED IN GRINDING, HONING AND POLISHING

1 *Soap Solution*—This is used in all steps of sharpening, with the exception of stropping. A level teaspoonful of pure powdered castile soap is added to a pint of boiling water in a one liter Erlenmeyer flask, and the mixture shaken thoroughly until the soap is completely dissolved. After cooling under a faucet, the solution is ready for use. All dust particles in flask or drip can must be removed by previous rinsing. The soap must be free of dust and impurities which might scratch.

2 *Emery Flour*—This abrasive is used for truing the knife edge and grinding out nicks. Only the best Turkish emery flour, FFF, is suitable. It was found that levigation, just previous to use, gave best results in that larger particles were avoided. About  $\frac{1}{4}$  teaspoonful of emery is added to a 20 cc test tube filled three-fourths with water. After thorough shaking the tube is brought to rest for a few seconds to allow coarser particles to fall to bottom. The upper  $\frac{7}{8}$  of the suspension is poured quickly on the revolving disc for grinding. It is better to discard the sediment.

3 *White Rouge*—This abrasive is a chemically prepared silicon dioxide used by lens makers for polishing optical glass, but becomes a quick abrasive

\*From the Laboratories of Pathology and Bacteriology, Medical School, Temple University, Philadelphia, Pa.

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for steel. A suspension is prepared, using  $\frac{1}{4}$  to  $\frac{1}{2}$  teaspoonful to 15 cc of water, shaken thoroughly in a 20 cc test tube. The suspension is creamy in consistency and color, and is directly poured on the revolving disc without levigation, in the process of coarse honing.

4 *Diamantine Powder*—Swiss diamantine powder No 1 is used to finish honing and to polish the honed surfaces. Diamantine is a crystallized preparation of boron. This powder must be used quite sparingly. An amount heaped over a  $\frac{1}{4}$  inch square surface (about 1 to 2 grains Troy) added to 15 cc of water in a 20 cc test tube and shaken thoroughly is sufficient for one application in fine honing. A smaller amount suffices for the first step

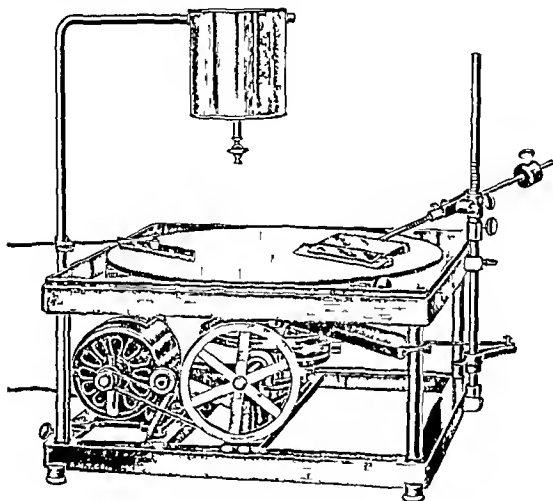


Fig 1—Automatic sharpener. Knife on attrition disk in position for grinding, honing and polishing.

in polishing. Final polishing is accomplished on the clean surfaced attrition disc, using nothing but the soap solution (100 to 150 drops per minute).

5 *Rouge*—Finely powdered polishing rouge (red iron oxide) is used for stropping. It should never be placed on the attrition disc but is dusted in small quantity on the paper stropping discs.

#### INSPECTION OF THE KNIFE TO DETERMINE PROCEDURES TO BE FOLLOWED

The edge should be examined throughout its entire length with a microscope or with a high power magnifier. The  $\frac{7}{8}$  objective with 10x ocular is the best combination (using reflected light). Great mistakes are those which involve all or most of taper length. These necessitate longer grinding with emery suspension from three fourths to two hours depending on the hardness of



the steel. Usually the nicks encountered in a knife which has not been abused are smaller, involving but a portion of the taper surface. These usually yield in from twenty minutes to one hour. Slight nicks and burred edges involve but very little of the taper surface, and are visible to the naked eye only on careful inspection when good illumination is provided. Under the microscope these are readily discernible as serrations and bendings of the edge. Diamond or white rouge honing without grinding will usually remove these slight imperfections, which, however, are so prohibitive to good sectioning. Knives, which present a belly at middle from repeated stone sharpenings, require

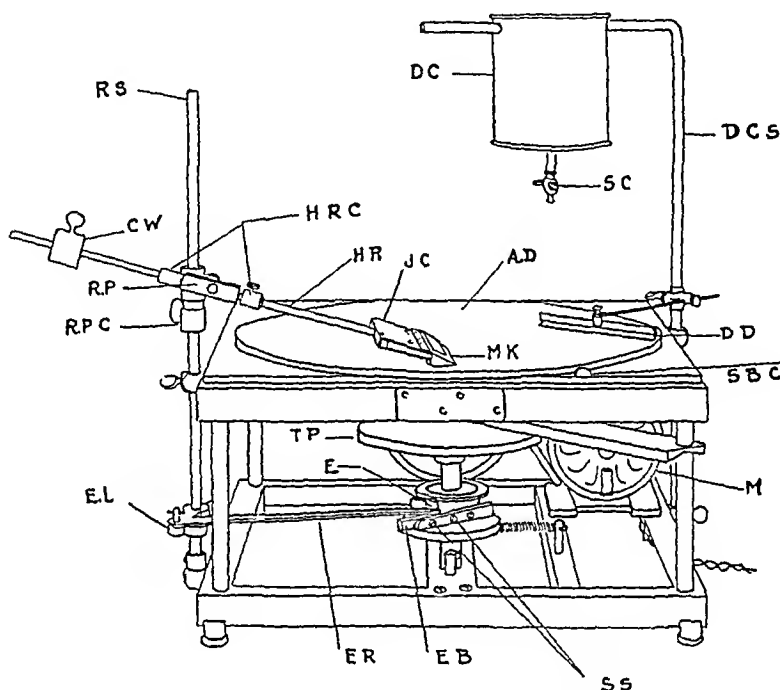


Fig 2—Automatic sharpener. Parts labelled for identification. Note knife on attrition disc is now in position for stropping. Paper disc not shown. (Drip deflector DD is not used in process of stropping.)

DC, drip can  
SC, stopcock  
DCS, drip can support  
DD, drip deflector  
RS, reciprocating shaft  
CW, counterweight  
RP, rest place on reciprocating shaft  
RPC, rest place collar  
HRC, handle rod collars  
JC, jaw clamp of knife holder  
HRC, handle rod of knife clamp  
MK, microtome knife

AD, attrition disc  
SBC, spring brass clip of which three grip the disc  
TP, transmission speed reducing pulleys  
E, eccentric  
ER, eccentric rod  
EB, eccentric block  
SS, set screws to fasten eccentric block in any position  
EL, eccentric lever on reciprocating shaft  
M, motor

periods of emery grinding from two to three hours or more, dependent on depth of belly and hardness of the steel. In these belled knives the disc must cut down each end of the knife edge before sharpening at center is possible.

#### EMERY GRINDING TECHNIC

By means of a carborundum pencil one side of the knife is arbitrarily marked "T" for top, and henceforth must be placed always in the holder to correspond with its top side (marked "T"). Center the knife in the holder. Screw down the two binding screws of clamp so that the knife is held firmly

Next, wash disc thoroughly under faucet with running water to remove all dust particles, and place same on the carrier head. Lay the knife holder handle in the rest on the reciprocating shaft. Adjust the holder so that edge of knife lies in the near radius of glass disc. With holder in this position, tighten thumb screws on each of the two collars of handle, one collar being set below, the other above the rest piece. (See H R C Fig 2) See that both collars are in contact with their respective end of rest piece (R P). To govern the length of tapering surfaces to be ground the rest piece is raised or lowered on the reciprocating shaft. By tightening the wing screw of rest piece its position is maintained. To facilitate this adjustment a collar below the rest piece is furnished (R P C) and also provided with a wing screw. It is obvious that by elevating rest piece and collar one will grind a short taper with a more obtuse edge angle. By lowering rest piece and collar the tapering surfaces forming the edge become longer the edge angle becoming more acute. For best sectioning results the tapering surfaces should be about 0.5 mm long, in fact our best knives on measuring with an eyepiece micrometer showed tapers of 0.55 to 0.57 mm long.

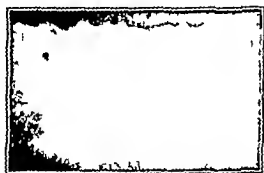


Fig 3—Edge of microtome knife after use in sectioning prostatic tissue containing calcific concretions. Not fractured and serrated edge.

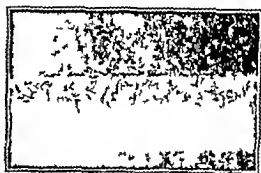


Fig 4—Same knife edge after coarser nicks have been removed by grinding 30 minutes with emery flour FFF.

Having adjusted the knife holder for radius position and taper length, the reciprocating movement must be regulated so that one end of the knife reaches the disc edge in the peripheral excursion the other end almost arriving at disc center on the centripetal excursion or swing. This adjustment is made by shifting the eccentric block and then fastening same by means of two set screws (see SS, Fig 2). Before turning on power place at least a pint of soap solution in the drip can and flood the disc. The power is turned on by means of a switch conveniently placed on the power cord. The disc rotates contra clockwise moving toward the knife edge which lies always in the near radius for grinding, honing, and polishing. Finally, set the drip deflector (see DD, Figs 1 and 2) in light contact with the disc wet with soap solution in such position as to conserve abrasive and soap solution and quickly pour on the emery suspension. The knife is turned in the rest piece without stopping the mechanism by depressing and rotating the handle rod. Two or three minutes is allowed each side before turning the knife over. The soap solution drip is regulated by stopcock under the drip can (see SC, Fig 2). From 100 to 150 drops per minute suffice to keep disc from drying. The abrasive suspension is replenished as required. It is a good policy to remove

\*Figs 3 & 4—Photomicrographs of all microtome knife edges made with 4 mm objective and 8x ocular reproduced without retouching.

knife holder containing the knife and to wash and examine the knife every fifteen minutes. The knife should never be removed from jaw clamp until sharpening is fully completed in the stage of polishing or stropping. The grinding must continue until the worst nicks have been completely removed. The fine uniform serrations visible on microscopic examination will remain and further emery grinding will not improve the situation, but in the next step of honing the serrations (provided they are uniform) disappear to give place to the much desired straight line edge (see Fig 4). The important precautions to be taken in emery grinding are

- 1 See that all large nicks are removed
- 2 That belly of the knife is trued



Fig 5—Same knife showing effect of grinding with white rouge after the grinding described in Fig 2. Note straight line edge and long bevel i.e. double the width of that usually found in a new knife such as shown in Fig 8. Surfaces converging to edge have not yet been polished.



Fig 6—Same knife showing edge after polishing on the glass disc of automatic machine with diamantine. Note high polish and long bevel affording unusual strength to cutting edge.



Fig 7—Knife edge similar to that shown in Fig 1 after hand honing and hand stropping by a trained hand. Note wavy edge which while lacking coarse serrations is worthless for sectioning tissue.

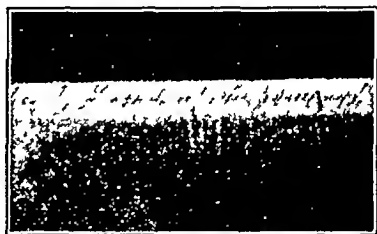


Fig 8—Edge of new knife as ground and hand honed by instrument maker. Note narrow blunt double bevel and coarse crevices terminating in a serrated edge.

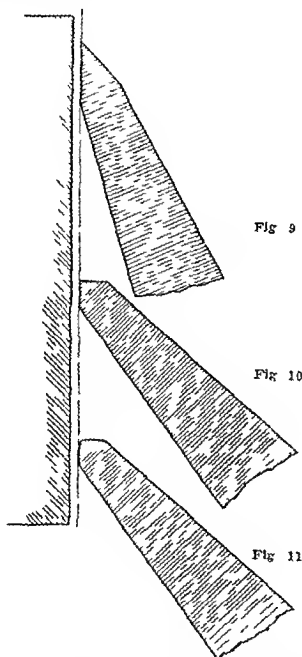
3 That the tapered surfaces converging to terminate in the edge are at least 0.5 mm long

4 That the grinding is uniform over the entire length of the knife, i.e., width of taper is the same throughout

#### HONING

After the process of grinding has been accomplished, the knife (still in its holder) is thoroughly flushed under a faucet, to rid it and the jaw clamp of all emery particles. The glass disc must also be removed and thoroughly flushed and wiped with a soft cotton towel. The disc is then returned to the carrier head (same side always up). Return the knife holder to its rest, without changing position of same. Flood disc again with soap solution and turn on the power. Readjust the dip deflector and add white rouge suspension

from the test tube, prepared according to directions found in section on "Materials" Five to ten muls, are deposited on disc each time, the disc having been previously wet with soap solution The knife must be turned every two minutes As honing continues, the white rouge gets darker due to steel particles ground from the knife It is well to flush the plate and knife with water every ten minutes to rid the disc of steel particles which mar the finish If this should not suffice a cotton towel wet with ether will entirely remove the stains of steel glaze The soap solution must be continued to prevent drying of the disc Examine the knife edge every fifteen minutes with either microscope or magnifier



SCHEMATIC CROSS SECTIONS OF UPPER PORTION OF MICROTOME KNIFE TO SHOW LENGTH OF TAPER, I CLINATION OF KNIFE TO BLOCK MULTIPLE BEVELING ETC

Fig 9—Knife sharpened on new apparatus Note acute ness of angle at cutting edge Compare with Fig 10 Knife so sharpened permits of its use at a more acute inclination to block surface than knife in Fig 10

Fig 10—Knife well sharpened by instrument maker Note angle at cutting edge (more obtuse), making a wide inclination of knife to block surface necessary This wide angle of inclination tends to make the knife scrape rather than cut

Fig 11—Knife apparently well sharpened judged by gross visible appearance but not satisfactory for sectioning Angle of cutting edge the same as in Fig 10 excepting microscopic rounding of the two planes forming the cutting edge This knife is without nicks and considered sharp by usual criteria The rounded edge makes necessary a still wider inclination of the knife to block surface If the same angle as in Fig 10 is retained the edge of the knife retreats from cutting position Knives so sharpened skip sections The rounded edge is usually the result of excessive stropping or careless hand honing

Add water to make 100 cc and treat the precipitate as above, after which 0.10 cc of the precipitate are dissolved in 20 cc of absolute methyl alcohol.

All of the precipitates obtained in this manner are not equally insoluble in water. The methylene blue-eosin precipitate may be washed 3 or 4 times with decomposition and it is preferable to treat it in this manner, but the precipitates obtained with the other dyes dissociate in water if the lavage is prolonged. These precipitates should not be washed more than once.

The staining is done in dishes from which all traces of acid or alkali have been removed by rinsing with distilled water. To 10 cc of distilled water there are added 25 drops of the toluidin orange or 5 to 7 drops of the other solutions. Two or three drops of a 1 to 1,000 solution of acetic acid are then added. The section is stained for twenty-four hours. After the excess water has been removed the section is washed with alcohol rapidly three times and then placed in xylol and mounted in balsam.

The success of the staining depends upon the degree of acidity of the bath, one drop of the diluted acid more or less will modify the result. If the bath is too acid the eosin and orange alone will stain while if it is too alkaline only the blue will stain. The number of drops of the diluted acetic acid can be obtained for each preparation after a few trials. The stain should never be filtered as filtration removes its activity, the greater part of the dyes being absorbed in the filtering.

This method of staining gives particularly good results for the study of lymphoid and splenic tissue fixed in Zenker's solution, and it is also successful for sections fixed by other methods. The preparation is never destroyed, if staining does not occur in twenty-four hours, it suffices to wash it with alcohol and distilled water and then the stain may be used again after changing the acidity.

#### **BACTERIOPHAGE** A Method for the Study of Bacteriophage Multiplication in Broth, Burnet, F. M. Brit J Exper Path 10 No 2, 109, 1929

A freshly made filtrate from a culture lysed overnight is titrated by plating successive dilutions (usually tenfold) with the sensitive strain on agar. The plates are incubated at 37° C until the plaques are clearly visible (five to six hours usually). From the plaque count the volume of the filtrate containing one phage particle is calculated, and a suitable amount added to 10 cc of a growing broth culture (four hours) of the sensitive organism to give one particle for each 0.03 cc of the culture. After thorough mixing volumes of 0.02 cc are removed in capillary pipettes previously calibrated. The pipettes are sealed at the capillary ends and placed in a water bath at 37° C. At the same time an effort is made to maintain the tube containing the mixture of phage and culture at about 37° during manipulations. As soon as the required number of pipettes have been filled, 40 or 60 in most experiments, 10 lots of 0.02 cc are gently blown out on to an agar surface, spread over a reasonable area by tilting the plates, and dried off as quickly as possible in the incubator. At suitable intervals batches of ten sealed capillaries are opened and their contents spread similarly.

#### **TUBERCULIN REACTION** Lovett, B. R. Am J Dis Child 37 No 5, 918, 1929

Attention is called to the value of the simple percutaneous method described below.

The skin over the sternum is rubbed with ether until hyperemia occurs. A piece of the concentrated old tuberculin, the size of a pinhead, is vigorously rubbed in with the finger tips. The result is read at the end of forty-eight hours. Usually the reaction appears within twenty-four hours, but occasionally it is delayed for three or four days, thus corresponding to the torpid reaction described by Pirquet.

Three grades of positive reactions may be described. The weakest consists of several small scattered papules at the site of inoculation, with scarcely any reddening. A moderate reaction is shown by a larger group of papules, with definite reddening. In the strong reaction a marked folliculitis appears, with minute vesicles or even pustules and intense inflam-

mation There may be some itching The milder reactions persist for several days the stronger may be visible for a few weeks Possible but rare sources of error are diffuse reddening or punctate reddening of the pores without folliculitis as may occur in a sensitive skin For a positive test there must be definite papules

**ENDAMEBA GINGIVALIS** Cultivation of Kofoid, C A and Johnstone H. G. Am J Pub Health 19 No 5 549, 1929

#### SALT SOLUTION

Sodium chloride	360 gm
Calcium chloride	8 gm
Potassium chloride	16.8 gm
Water	2000 cc

Sterilize in autoclave for thirty minutes at 15 pounds pressure

#### SODIUM BICARBONATE SOLUTION

Sodium bicarbonate	8 gm
Water	100 cc

This solution must be prepared carefully to prevent absorption of carbon dioxide from the air and it must not be heated or shaken vigorously The solution is filtered through a fine Berkefeld candle

#### SUGAR SOLUTION

Dextrose	25.1 gm
Water	100 cc

The sugar solution is sterilized in the Arnold for twenty minutes daily on three successive days

The salt solution is put up in 110 cc amounts the bicarbonate in 5 cc amounts and the sugar solution in 10 cc amounts The flasks of salt solution and the tubes of dextrose are capped with paper while the tubes of sodium bicarbonate are corked and covered with paraffin

To make 2 liters of Locke's solution take a flask of salt solution (110 cc), a tube of sodium bicarbonate solution (5 cc) a tube of dextrose solution (10 cc), and add to 1885 cc of sterile distilled water The above solutions are made every three months and can be kept indefinitely Two liters of Locke's solution can thus be prepared in about five minutes which is of great advantage when needed in a short time or in cases of emergency

**Locke's albumen solution** The white of one egg is carefully cracked into a sterile liter flask containing glass beads shaken vigorously until frothy then 500 cc of Locke's solution and 20 cc of N/20 HCl are added This quantity of acid proved to be about the right amount to add to a liter of fluid in order to give a  $P_{H}$  7.4 to 7.6 The remaining 500 cc of Locke's solution are added and the whole mixture shaken thoroughly and filtered through a Berkefeld candle This sterile medium is added aseptically in 6 to 10 cc amounts to the egg slants

It is possible to make the Locke's egg albumen medium without filtering but it has been the experience that the filtered medium gives by far the most satisfactory results The unfiltered medium is prepared exactly as the filtered with the exception that 2 egg whites instead of one to a liter of Locke's solution are used The eggs must be sterilized in 90 per cent alcohol before cracking and aseptic precautions observed to avoid contamination An un inoculated control tube should be run at the time of each transplant

The medium is warmed to 37° C previous to inoculation with either fresh or culture material Subgingival exudate or pus is removed by sterile sharpened applicator sticks or

sterile dental scalers, and introduced into the culture tubes. The tubes should be placed immediately in the incubator at 37° C and at no time should they be subjected to prolonged chilling.

Cultural transplants are made by removing a small amount of the material which collects in the angle formed by the egg slant and the side of the tube with a sterile Pasteur pipette and inoculating this substance into fresh medium warmed to 37° C. Transplants are made every forty eight hours.

### BLOOD The Volume and Hemoglobin Content of the Red Blood Corpuscles, Wintrobe, M M Am J Med Sc 177 No 4, 513, 1929

The following formulae for the calculation of the "corpuscular volume" are presented

$$\frac{\text{Red cell volume in cc per 1000 cc of blood}}{\text{Red cell count in millions (and fractions) per cmm}} = \text{corpuscular volume in cubic microns (10 to 12 cc)}$$

$$\frac{\text{Hemoglobin (gm per 100 cc)}}{\text{Red cell count (millions per cmm)}} = \text{"corpuscular hemoglobin"}$$

The actual proportion of the substance of each red cell taken up by hemoglobin may be calculated by dividing the corpuscular hemoglobin by the corpuscular volume, or, directly, by dividing the hemoglobin content of a given sample of blood, expressed in grams per 100 cc, by the total cell volume expressed in cc per 100 cc.

The example illustrates the calculations

A sample of blood is found to contain 5.85 million red cells per cmm, 15.87 gm of hemoglobin per 100 cc (or 158.7 gm per 1000 cc) and 46.5 cc of packed red cells per 100 cc of blood (46.5 cc per 1000 cc). Then, the average corpuscular volume is  $\frac{46.5}{5.85} \approx 7.95$  cubic microns (or  $7.95 \times 10^{-12}$  cc), the average corpuscular hemoglobin is  $\frac{15.87}{5.85} = 2.71 \times 10^{-12}$  gm, proportion of hemoglobin in the average cell is  $\frac{2.71}{7.95} \times 100 = 34.1$  per cent (or  $\frac{15.87}{46.5} \times 100 = 34.1$  per cent).

It is obvious that the methods used for the preliminary determinations must be accurate.

### GRAM STAIN Modification of, Zeissig, A Stain Technol 4 No 3, 91, 1929

- 1 Apply nuclear stain
- 2 Wash
- 3 Stain in Hucker's gentian violet two to three minutes (i.e. 1 part saturated alcoholic solution crystal violet to 4 parts 1 per cent aqueous solution ammonium oxalate)
- 4 Wash in water
- 5 Stain in Gram's iodine five minutes
- 6 Wash in water
- 7 Decolorize in 95 per cent alcohol to which enough tincture of iodine has been added to give a mahogany color
- 8 Counterstain
- 9 Dehydrate and mount

### PEROXIDASE REACTION A Modification of the Peroxidase Reaction, Etc, Strumia, M M Arch Path 5 No 3, 447, 1928

The following modification can be used not only for blood smears but also for frozen sections (formalin fixed)

Two stock solutions are prepared

Solution A Sodium nitroprusside, 5 per cent aqueous solution

Solution B Benzidine, 2.5 per cent alcoholic solution

For use, mix 1 cc of Solution A and 95 cc of 95 per cent alcohol, add 2 cc of Solution B and 2 cc of fresh solution of hydrogen peroxide.

This solution must be allowed to ripen for an hour or more to give the best results. While this mixture keeps at least as well as the original Goodpasture solution, the stock Solutions A and B keep indefinitely; the mixture can easily be prepared each time it is needed by mixing the stock solutions in proper proportion.

The technique of staining is the same as in the original Goodpasture method. The dried blood smear (which should be fresh) is covered with the solution for one to two minutes. An equal amount of distilled water is then added and the preparation is stained for from three to five minutes until under the low power of the microscope blue granules appear in the polymorphonuclears and large mononuclears. The slide is then washed in running water for about fifteen minutes.

#### TULAREMIA A Presumptive Test for Hull, T. G. Am J Pub Health 19 4 423 1929

##### CULTURE MEDIUM FOR BACTERIUM TULARENSE BLOOD GLUCOSE CYSTINE AGAR

Fresh beef infusion agar containing 10 per cent peptone, 15 per cent agar, and 0.5 per cent sodium chloride adjusted to a  $P_H$  of 7.3 is kept on hand in stock. When needed there is added to the stock agar 0.5 per cent of cystine and 10 per cent glucose and this is heated in an Arnold steam sterilizer sufficiently long to melt the agar and to sterilize the cystine and glucose after which it is cooled to 40 to 45° C. When 5 to 8 per cent defibrinated rabbit blood is added. Overheating and loss of the bright red color should be avoided. The medium is then tubed, slanted and incubated to insure sterility.

The addition of the cystine does not change the  $P_H$  of the medium but if cystine hydrochloride is substituted for cystine a correction may be necessary on account of acidity. Cystine is not very soluble in the beef infusion peptone agar and for that reason it should be pulverized before being added; even then visible particles settle in the medium.

Tubes in which the water or condensation has evaporated are preferable. The organism scarcely grows in a liquid medium. Freshly prepared tubes with moist surface and with abundant water of condensation should be allowed to stand with cotton stoppers in a slanted position, in the incubator at 37° C. for about one week until almost free of water of condensation. They are then stood upright and plugged with cork stoppers soaked in a very hot mixture of 1 part vasoline and 2 parts paraffin to prevent further evaporation; then they are stored in the cold room.

Stock cultures are kept in the cold room and transferred every two months. Cultures are stoppered with cotton during two or three days of incubation but with paraffin cork during two months in the cold room at 4 to 10° C. preferably 4° C.

A large loopful of growth is carried over when making transfers. A control tube of plain agar should show no growth.

The blood in the medium conduces to luxuriance of growth and to longevity of life of the culture. 8 per cent is preferable to 5 per cent. Human blood might be more accessible than rabbit blood and might serve equally well.

##### MAKING THE ANTIGEN FOR AGGLUTINATION TESTS

Blood is omitted from the medium when growing antigens for agglutination or absorption tests. The medium then becomes glucose cystine agar.

Blake bottles containing glucose cystine agar are employed when a large quantity of antigen is desired. Before being inoculated the bottles should dry several days in an inverted position in the incubator and any water of condensation should be pipetted off before inoculation. Each bottle is inoculated with the entire growth from a blood glucose cystine agar slant suspended in 1 cc of physiologic saline solution. This suspension is quickly spread over the surface of the medium in the Blake bottle by rocking the bottle in the hands, all excess of fluid being absorbed by the dry medium.

Incubate the bottles in their normal noninverted positions at 37° C. for three days. Take off the growth of each bottle in 1 cc of physiologic saline solution containing 0.2 or 0.3 per cent of formalin (U.S.P. strength 3 per cent). Throw down the bacterial mass in the centrifuge thereby washing the organisms. Pour off the fluid. Take up the bacterial



sterile dental scalers, and introduced into the culture tubes. The tubes should be placed immediately in the incubator at 37° C and at no time should they be subjected to prolonged chilling.

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$\frac{15.87}{5.85} = 2.71 \times 10^{-12}$  gm, proportion of hemoglobin in the average cell is  $\frac{27.1}{79.5} \times 100 = 34.1$

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## REVIEWS

Books for Review should be sent to Dr Warren T Vaughan, Medical Arts Building  
Richmond Va

### Old Age\*

THIS arresting essay is reviewed in the editorial pages of this issue

### Serodiagnosis of Syphilis†

THIS second report from the Health Organization of the League of Nations records and analyzes the results of 944 tests by various methods, the technique of which is presented in full in an appendix to the report

The methods used during the conference were

1 Complement fixation tests of which there were several varieties (a) That known as Method No 1 of the British Medical Research Council (b) The Blasch modification (c) The method of Calmette and Massol modified by Debquins (d) The synoptic method of Jacobsthal (e) The original Wassermann (carried out by German and Serbian workers), and (f) The method of McIntosh and Fildes (as used by Polish workers)

2 Flocculation Tests The Kahn reaction, Meisacke's Turbidity Test (MTR) Muller's Clotting Test (MCR) Murata reaction Sachs-Georgi (Cito and Lentochole reaction) the Sigma reaction (modified) and the Vernes reaction

All the tests mentioned are described in detail in the appendix a section of some interest to all who have followed or been engaged in the evolution of serologic technique

It seems somewhat surprising to the reviewer to note the emphasis laid upon certain phases of technique in the modification of the Wassermann test, the principles of which have been more or less generally recognized for nearly a decade by American serologists to note the extent to which the original Wassermann technique still holds sway in Europe with relatively minor modifications—Otto and Blumenthal for example use as antigen an extract of syphilitic liver—and, in general, the absence of many of the technical refinements based upon the studies of American serologists. It would appear in fact, that the studies of American serologists in the last decade have been rather generally overlooked or ignored by European investigators

In a comment which appeared in this Journal (January, 1926) upon the first report of the League of Nations upon serodiagnosis in syphilis it was said "It is difficult to avoid the impression that there was an effort to show that each of the methods was an excellent method in the hands of its originator or proponent" The second report likewise carries a suggestion of the same impression

The details of the report are of interest to all engaged in serologic examinations Kahn, for example suggests a still further modification in the method of precipitating the globulin from spinal fluids for examination (use of small tubes, 7.5 by 1 cm and 50 per cent instead of 40 per cent ammonium sulphate solution) and Col Harrison comments specifically upon the unsuitability of the Kahn test for use with cloudy spinal fluids while Professor Vernes comments upon the fact that the conference depended upon +, -, and ±

Old Age. The Major Involution. The Physiology & Pathology of the Aging Process. By Aldred Scott Watlin PhD MD LL.D Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan Ann Arbor With 9 Illustrations Pages 200 Hoeber New York 1929

†Report of the Second Laboratory Conference on the Serodiagnosis of Syphilis. League of Nations Health Organization Geneva 1928 Cloth p 185

NOTE In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed and (b) description of the contents so that the reader may judge as to his personal need for the volume

We trust that the scientific information printed in the foregoing pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto

results As he said "It was through sustained clinical observation that we were led nearly twenty years ago to the fundamental conclusion that serology would be condemned to remain a rudimentary science if it could only produce + or - results, while in clinical practice and all other laboratory sciences, *fluctuations* are observed and noted At the conference, however, in order that our results might be compared with others, we were asked to express them by the symbols +,  $\pm$ , and -, which we thought we had buried forever when we established our first scale in 1910 "

Perhaps in the future, once the question of relative specificity is settled to the satisfaction of the Conference, attention to the vitally important matter of *quantitation* of serologic reports will be studied

It is to be hoped that from some future conference will come a determination of the best available European method for the conduct of the complement fixation test, for then it can be compared with the more outstanding American methods to the great interest of all concerned

The conclusions of the Conference are of decided interest, so much so as to warrant extended quotation

It is of the opinion that "the best of (the flocculation tests) are equal in value to the best of those which depend upon the fixation of complement "

The Conference "desires, nevertheless, to emphasize the fact that, no less than the complement fixation tests, these flocculation methods are, despite their apparent simplicity, extremely sensitive to the slightest differences in experimental conditions and subject to so many sources of error, in connection both with the execution of the test and the reading and interpretation of the results, that they must be placed only in the hands of specially trained serologists "

This conclusion is of especial interest in view of the agitation following the introduction of the Kahn test for its *exclusive* use in the serologic study of syphilis

In this connection, 17 of the 38 workers at the Conference express the opinion that "(a) As theoretical considerations give reason to expect, some sera react to the Bordet Wassermann but not to the flocculation tests and vice versa, and that the Bordet Wassermann and the flocculation tests supplement each other, (b) That strong confirmation of a weak or  $\pm$  flocculation test is afforded by a positive Bordet Wassermann test and vice versa,

" these workers "prefer that one of the methods used should be a Bordet Wassermann test "

The Conference expresses the opinion that "in order to secure the most reliable information to the clinician, at least two different serodagnostic methods should be used, to which the paragraph above is an addendum

"It is a matter for some surprise that the Conference suggests a return to merely plus and minus reports when in view of the many studies, it may be doubted will assist to the fullest extent clinicians in observing progress under treatment "

Serologists in general will be most heartily in accord with the following "The Conference desires to record its view that considerable misunderstanding would be avoided and reports on tests of sera would be greatly enhanced in value if clinicians would study closely the diagnostic and therapeutic implications of such reports "

### *The International Medical Annual\**

THE fact that medicine is an ever changing art has had within recent years a noteworthy effect upon the character of medical books

The ponderous, encyclopedic "systems" of yesteryears have given way in no small measure to smaller and more flexible texts which, in turn, are supplemented by the monographic contributions of specialized authorities

The newer advances, the trials, failures, and successes of new methods and the modern adaptation of older ones appear, however, in the relatively ephemeral journals Unless one

\*The International Medical Annual 1929 A Year Book of Treatment and Practitioners Index. Edited by C F Coombs M.D. and A R Short M.D. Cloth 568 pages 71 plates and 89 text figures William Wood and Co New York

has access to, and time to read, all or at least the greater majority of these it will not be long before, to some extent he will fall behind the times

To meet just such a situation even more acute now than it was then the International Medical Annual was begun forty seven years ago. The present volume, the work of thirty one contributors presents in an eminently readable manner a comprehensive yet succinct summary of the advances made during 1928 in the treatment of disease by all the many means comprised in the generic term 'the practice of medicine'

The material thus reviewed digested and summarized is presented in a most practical manner as in "Dictionary of Practical Medicine"

Each subject is arranged in alphabetical order and there is moreover, a very complete index and cross index so that the particular item desired may be readily found

Although the work of English authors the catholic character of the book is evidenced by the cosmopolitan array of the sources from which the reviews have been compiled. Not only the literature of Europe but the literature of the United States is well represented in the survey of the advances of the year

Interspersed throughout the book are original contributions of practical value. The illustrations are excellent in every way

For the practitioner at large this is an exceedingly practical and useful volume well deserving of a handy niche in the office where it can be conveniently and frequently consulted

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Editor WARREN T VAUGHAN, M D

Richmond, Va

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## EDITORIALS

### Medical Mysteries of the American Wilderness

WITHIN the wildernesses of the earth have always lurked many of man's deadliest enemies. While Moses and the Israelites were eking out a hungry and disconsolate existence in the wilderness of Sinai we read that "there went forth a wind from the Lord, and brought quails from the sea, and let them fall by the camp, as it were a day's journey on this side, and as it were a day's journey on the other side, round about the camp, and as it were two cubits high upon the face of the earth.

"And the people stood up all that day, and all that night, and all the next day, and they gathered the quails. He that gathered least gathered ten homers, and they spread them all abroad for themselves round about the camp.

"And while the flesh was yet between their teeth, ere it was chewed, the wrath of the Lord was kindled against the people, and the Lord smote the people with a very great plague.

"And he (Moses) called the name of that place Kibroth hattaavah because there they buried the people that lusted

"And the people journeyed from Kibroth hattaavah unto Hazeroth, and abode at Hazeroth '

While in this instance as usual Moses and the Lord operated on a scale of bewildering magnificence, yet in the more modest circumstances of recent times many of the features of this early plague have again manifested themselves. The primitive forests streams swamps prairies and deserts of the early United States constituted an excellent setting for a number of the pestilences which have affected man or beast or sometimes both. While in the beginning profound mystery surrounded all these plagues which had their origin in the wrath of the Lord or in some similar etiology yet one by one most of these mysteries have yielded to the eager inquiring spirit of the modern epidemiologist and research worker.

Of the early American epidemics perhaps none was more mysterious or stealthy in its approach than was the malady known as "milk sickness" or the "trembles." Already known to the Indians this disease continued like an hereditary scourge to blight great sections of the territory lying roughly west of the Allegheny mountains east of the Mississippi River northward to the Canadian border and southward to Georgia and Alabama. Accounts of the disease were left by Barhee in 1809 and by Telford and Stewart<sup>2</sup> in 1812. But the first comprehensively accurate and complete account of the malady was given by Daniel Drake<sup>3</sup> in 1841. Drake made a series of long and dangerous journeys throughout a large section of the affected region. While he personally saw no cases of the disease still he collected all the data that he could possibly obtain from the natives and the physicians of the region (several of whom had attended many cases) and he made an extensive study of the botany, the geology and of the climatic conditions of the country. The disease attacked both animals and human beings apparently being transmitted to the latter by the drinking of milk from affected cows or from the eating of meat from animals having the disease. Drake listed eight animals as being subject to the disease, viz. the cow the horse the sheep, the hog and the dog, to which he added as doubtful, the goat, the mule, and the buzzard. The treatment included blood letting, cathartics, opium, counter irritants, cold affusions antacids alcoholic tinctures and other 'diffusible stimuli' drinks such as a coffee made of scorched oats, and infusion of wheat or weak chicken broth.

Regarding the etiology of the disease Drake, after making many observations and considering many possible origins finally announced his conclusion that the disease was caused by the *Rhus toxicodendron* which was eaten by the cattle. That was in 1841. It is interesting to note that Beach<sup>4</sup> writing in 1887 was able to summarize the accumulated knowledge of his day on the subject by stating that "first there are the theorists who cling to the idea of ingestion, by the herbivora, of some injurious form of vegetation, second, those who attribute it to a deleterious water supply third, the marsh miasmatic theorists, fourth, those who attribute it to some mineral poison that has an affinity for moisture, and, rising with the dews, settles on and

adheres to vegetation, or, rising with the dews, is taken in by inhalation, fifth, those who believe that its cause is bacterial—that spores, bacteria, or some microscopic fungi or disease germs are taken into the system with either food, the water, or the inspired air ”

These suggestions obviously represent the 1887 version of the “wrath of the Lord ” And while various writers<sup>6</sup> of the present day have considered the disease to be caused by *Eupatorium utricac folium* (white snake root), Jordan and Harris,<sup>7</sup> on the other hand, in 1907 found no white snake root in certain regions where the disease prevailed, and they considered it to be due to the *Bacillus lactimorbi*. And Baldwin<sup>8</sup> writing in 1927 found it necessary to state that “the specific etiology is in doubt ” It is worth while to reflect that during a large part of the last century it was often necessary for the settlers in affected regions to give up and desert large tracts of valuable land on account of the disease. In fact, they were still “journeying from Kibroth-hattaavah unto Hazeroth ”

Another disease of somewhat similar character and history is the “loco disease,” or “locoism ” A considerable number of plants have been considered as causes of this disease. Kramer<sup>9</sup> mentions the California loco-weed (*Astragalus crotalariae*), the Texas or woolly loco-weed (*A. mollissimus*) and rattle-box (*Crotalaria sagittalis*). He also states that “*Clytonia glycinoides* of Brazil, *Phaca ochroleuca* of Chile and *Oxytropis lamberti* of Mexico are poisonous to horses and should probably be included with the loco-weeds ”

Following up the investigations of a number of other excellent workers<sup>10</sup> in this field, Couch<sup>11</sup> has recently published an extensive series of very interesting experiments on locoism in which cats have been used as experimental animals. He has worked with an extract of *Oxytropis lamberti* and has succeeded in isolating from the extract a highly toxic fraction which will produce typical symptoms of the disease in cats.

Although Couch has not succeeded in isolating the loco-poison in a pure state he has obtained it sufficiently free from foreign substances to furnish some definite information concerning its nature. It is very soluble in water and extremely hygroscopic, less soluble in alcohol and methanol, and still less soluble in acetone. It is quite insoluble in ether, chloroform, and the hydrocarbon solvents. It is unaffected by boiling with dilute mineral acids or alkalis, which indicates that it is not of either ether or glucosidal nature. It does not reduce alkaline copper solutions and is not basic. No precipitant has been found for it. It does not affect the ray of polarized light and it is free from barium compounds.

Thus little by little the “wrath of the Lord” is being confined within narrower and narrower limits, and the necessity of fleeing from “Kibroth-hattaavah” to “Hazeroth” grows less and less.

#### REFERENCES

- 1 Numbers 11 31-35
- 2 Barbee. Notices Concerning Cincinnati, 1809
- 3 Telford, Alexander, and Stewart, Arthur. Medical Repository, N. Y. 15 92-94, 1812
- 4 Drake, Daniel. Western J. Med. & Surg., pp. 162-226, March, 1841
- 5 Beach, William Morrow. Reference Handbook of the Medical Sciences 5 9, 1887, Wm. Wood & Co.
- 6 Sollmann, Torald. Manual of Pharmacology, Philadelphia, 1926, W. B. Saunders Co., p. 194

- 7 Jordan, E O, and Harris, Macleod *J Infect Dis* 6 492 1909
- 8 Baldwin, Horace S Cecil Textbook of Medicine Philadelphia 1927, W B Saunders Co, p 333
- 9 Kramer, Henry Textbook of Botany and Pharmacognosy Philadelphia 1910, J B Lippincott Co, p 299
- 10 Crawford J A M A 50 458 1903  
     Ibid 51 1338 1908  
     Alsberg and Black Bull 246 U S Dept of Agriculture Part II 1914  
     Alsberg and Black J A M A 59 2258 1912  
     Marsh, C D U S Plant Ind Bull No 112, 1909  
     Marsh, C D 1912 Bureau of Plant Industry Bull No 246 Part I  
     Crawford, A C Bureau of Plant Industry Bull No 129 1908  
     Marshall, H T Johns Hopkins Hospital Bull 25 234 1914  
     Also Department of Agriculture Bull 380
- 11 Couch James F J Pharmacol & Exper Therap 36 55 1909

—D E J

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### The Aging Process

TO MANY the conception of personal immortality is a beautiful idea to be devoutly prayed for. To most of us the possibility of prolongation of existence on this earth preferably as a happy existence, offers more promise of an immediate fulfillment. The fountain of youth, whether it be a new supply of drinking water, a commercialized spa, a testicular transplantation, a vasectomy, an endocrine rearrangement, a new wrinkle in calisthenics, Metchnikoff's Bulgarian bacillus, or any of the thousands yet to be proposed, will always seem just around the corner. Even the great Bernard Shaw basing his ideas on a little biologic knowledge, overlooks the vital fact and in his "Back to Methuselah" tickles the palate of those hedonists who would stay young long and grow old gracefully. Probably he wrote it with his tongue in his cheek.

We hear much of the prolongation of the average life expectancy. True in the forty years from 1880 to 1920, twenty years were added to the average duration of human life. This is a wonderful achievement accomplished principally by the saving of infants and children and fortunately, it has carried with it increased comfort, health and happiness to those who are living longer.

But it is quite a different matter to talk about lengthening the individual life span beyond its present limitation of ninety to a hundred years. In the period from 1880 to 1920 the average life was longer and there was indeed a progressive increase in the number of persons reaching the ages from eighty to ninety. But above ninety there has been no increase whatsoever in the proportion of survivors.

In the last fifty years more folks have grown older but these older folks have been unable to add still more years to the maximum of a hundred and more years ago.

Of course we would like to live to a ripe old age and more particularly to maintain our health during the living. Thanks to the advances of the medical and allied sciences this goal is slowly being approached. But can we further cheat the grim reaper by widening the life span, say to 125 or 150 years? For the welfare of the race, would it even be a desirable development?

Aldred Scott Warthin thinks not. In a charming volume on old age Dr. Warthin presents his thesis on aging or as he calls it *the major involution*



Living matter is granted the lease of life as long as it has a function to perform and performs it. The function having been accomplished and completed, deterioration sets in. An unused elastic will lose its "life." A spare tire will rot more rapidly than one on the wheel. Man's function in a biologic sense is to reproduce his kind and then to live long enough to protect his offspring until the latter becomes independent and self-sustaining. After that, biologically, he has no further function to perform. It would be splendid to see the great minds live to an old age, helping direct the progress of evolution and retaining their brilliancy to the end. It is more than pathetic to see the once brilliant mind lose its luster, but continue to exist in a desultory fashion in a body that has lived too long. The vast majority of persons who today reach senility are dependent on others, usually their children, for continued existence. At the present stage of our evolutionary program the prolongation of the total life span would mean but the increasing of the number of senile dependents.

Will the prolongation of health and efficiency into the higher decades that is admittedly occurring today result in an extension of ability, efficiency and independence into what are now the senile years, with a consequent setting back of these latter into still higher decades? Warthin believes not. Indeed, in accordance with his theory of degeneration after completion of function, men in the future should live not quite so long, instead of longer, since nowadays the offspring reaches independence at a somewhat earlier age.

Whatever corroboration the future may or may not bring on this, Dr. Warthin's discussion of those involutionary changes which follow cessation of function is most illuminating.

Involution commences practically synchronously with fertilization. The spermatozoon once having penetrated the ovum, motility is no longer requisite, and the tail of the spermatozoon rapidly involutes. Both the ovum and the spermatozoon suffer a reduction in chromosomes, which means involution. Even in embryonic life involution keeps pace with evolution. The branchial clefts subserve a function in determining the conformation and arrangement of various organs and structures of the head region, and then disappear. The notochord which marks the transition from the invertebrate stage of the embryo to that of the vertebrate, later disappears. The embryonic tail becomes incorporated within the body of the coccyx. Of the several mammary glands which commence to develop, only the two breasts persist. These are only a few of the several embryonic organs which serve some function and then disappear. It is in the placenta especially that we first see involutionary processes which are later comparable to the involution of man himself. At birth the placenta is a senile structure presenting sclerosis and obliteration of arteries, fibrosis of stroma, atrophy and degeneration of the chorionic epithelium, thrombosis, infarction of villi, calcification, etc. "All of the evolutionary tissue changes of old age appear in this important vascular organ at the completion of the period of intrauterine life. In the fulfillment of its function the placenta passes through well-defined stages of evolution, maturity, and senescence, and when its work is over it is discarded as useless, and disappears from the scene of the individual's life."

"In the newborn child blood vessels which served a function during embryonic life but which, with the onset of respiration and after the severing of the umbilical cord, have no further function to perform, degenerate and become obliterated, very much as occurs in the general circulation in old age "

In the majority, or in all of the vascular involutions of the developmental period, the exciting cause of these premature senescent changes appears to be a *loss of usefulness, a reduced function and not overwork*. The characteristics of these early vascular changes are identical in kind with those of the arterio sclerosis of later life. The changes are associated with disuse rather than overuse. They are not wear and tear changes nor are they toxic. The thymus when it has completed its function whatever it may be, atrophies, with disappearance of parenchyma, obliteration and sclerosis of blood vessels, fibrosis and fatty infiltration.

Involuntary changes occur in the ovary after each menstruation, with resulting sclerosis and calcification. At the menopause the ovary as a whole is as distinctly a senile organ as was the placenta at full term. 'It is much reduced in size except for cyst formation irregularly scarred on its surface and fibroid in consistency. Microscopically it consists chiefly of sclerotic blood vessels and the remains of corpora fibrosa with here and there a cystic follicle "

These same vascular involuntary processes are so pronounced in the involuting uterus following pregnancy as to be of diagnostic value, where there is a question of preexisting pregnancy.

The essential tissue changes of all of these minor developmental involutions are the same as those that occur in the body as a whole with senility. They consist of parenchymatous atrophy and degeneration with vascular obliteration and sclerosis.

The minor involutions occur in specialized organs and tissues which have completed their function and are destroyed for the good of the individual. The major involution or senescence occurs in all of the organs and tissues because the individual has fulfilled his usefulness and it is time that he make place for others. The minor involutions take place for the good of the individual, the major involutions for the good of the species.

A man who lives too long is apt to hinder evolution rather than help it. It is by continuous creation of new forms of the same life better adapted to the ever occurring changes in the environment, that evolution in its broad sense occurs. "The universe, by its very nature demands mortality for the individual if the life of the species is to attain immortality through the ability to cope with the changing environment of the successive ages "

We are here on this earth, primarily not to perpetuate our individual existences but to perpetuate the species. The energy charge known as life is greatest in the original fertilized cell and from this instant there is a gradual but progressive diminution in energy charge up to and through old age. The life of the species is perpetuated by repeated renewals of the energy charge.

The energy charge is expended at a constantly diminishing rate through the period of growth and development until maturity is passed, after which it declines still more rapidly until the machine finally comes to a standstill. This termination of the activities of the protoplasmic energy machine due to loss of its intrinsic energy is normal or biologic death. Growth, that is, cellular activity, is greatest during the first month of intrauterine life. Growth during the second month is relatively greater than that from the period of birth to maturity, in the third month it equals relatively that of the first six years of extrauterine life, and in the fifth month it is as great as in the first year of life. After birth the growth energy is strongest during the first year. During this time height is increased about 50 per cent and weight about 200 per cent.

At maturity the balance between cell growth and cell destruction becomes equalized so that repair is possible without loss of tissue elements and the curve of *nutrition and metabolism* remains relatively level until the onset of senility, when the energy curve again drops off rather rapidly.

Fortunately for our peace of mind, according to Warthin the psychic function continues to evolve as a rule up to about seventy years, before it too involutes.

Normal biologic death appears to be rare. Disease and other catastrophes of our daily life usually terminate the process sooner. Premature involutionary changes are not infrequent. We might say that in such persons death is still biologic but that their normal life span is for some intrinsic reason shorter than the average.

This raises the question whether the therapist may hope to accomplish anything for the individual in the treatment of premature degenerative changes such as arteriosclerosis, myocarditis, nephritis, other than to the extent of protecting the individual by conserving his energy output. Must we admit defeat in the face of premature degenerative changes and inform the patient that he has fulfilled his function and must be prepared to continue on the downward physical path?

Or may we still cling to at least a remnant of the wear-and-tear hypothesis and try to lead the victim out of his predicament by early recognition, removal of infectious foci, prevention of toxic absorption, avoidance of metabolic overload and the like?

There is no gainsaying that some are predestined from birth and by inheritance to premature senile changes. Will wear and tear intensify this innate predisposition or is it exclusively a matter of functional involution? Dr. Warthin's thesis explains the changes of senescence. It enables us to understand the pathology of premature senescence. But does it explain the cause for premature senility? Is it entirely a matter of heredity or may it be influenced by extrinsic and intrinsic constitutional factors which enter into the picture after birth? Warthin does not answer this question although he does describe premature old age as an intrinsic pathologic cause of death rather than as biologic death. It is a question that none can answer at the present time with certainty but yet one of the utmost interest.

Certainly we would all agree with Dr Warthin in his views on rejuvenation. Gland transplantation and vasectomy may temporarily stimulate the sexual appetite but it will have no effect on the major involution and, like new wine in old bottles it may unfortunately be sufficiently stimulating to be responsible for fatal catastrophe. Like Warthin we would speak not of rejuvenescence but of reerotization.

## REFERENCE

Warthin Aldred Scott. Old Age The Major Involution. New York, Hoeber, 1929.

—W T V

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## THE 1929 WARD BURDICK RESEARCH AWARD OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS TO

WALTER MALCOLM SIMPSON, B S , M S , M D , DAYTON, OHIO

THE first Research Committee of the American Society of Clinical Pathologists in 1928 planned a fitting memorial to one of the founders and the first Secretary-Treasurer of the Society by the institution of the Ward Burdick Research Award in the form of a gold medal bearing a profile image of the late Dr Burdick on one side and the Society seal and name of the recipient on the reverse

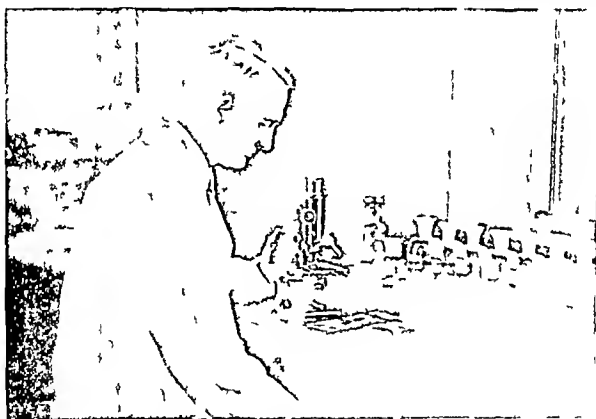
At the Eighth Annual Convention held in Portland, Oregon, in July of this year, the first medal was awarded upon decision of the Research Committee, by the President on the evening of the Annual Banquet to Walter Malcolm Simpson for his original studies on the subject of *Tularemia*

Dr Simpson, a graduate of the University of Michigan, was born December 25, 1895, in Lynn, Massachusetts. At the outbreak of the World War he enlisted as a seaman in the United States Navy and was soon advanced to the rank of Ensign and attached to the U S S *Arkansas* of the Atlantic Fleet as a watch and gunnery officer. In February, 1919, he returned to the

University of Michigan to complete his premedical studies and received the degree of Bachelor of Science in 1922, Master of Science in Pathology in 1923, and Doctor of Medicine in 1924

From 1920 to 1923 Dr Simpson was a teaching assistant in anatomy, histology and pathology in the University of Michigan, from 1923 to 1924 he was instructor in pathology and from 1924 to 1926 was senior instructor in pathology at the University of Michigan. In 1926 to 1927 he was instructor in surgical pathology in Johns Hopkins University and since 1927 has been Director of the Diagnostic Laboratories of the Miami Valley Hospital of Dayton, Ohio

Dr Simpson is a Fellow of the American Society of Clinical Pathologists and the American College of Physicians, a member of the American Medical Association, Montgomery County Medical Society Michigan Academy of Sci



ence, American Association of Pathologists and Bacteriologists Ohio Academy of Science and the Central Society for Clinical Research

At the 1928 meeting of the American Medical Association in Minneapolis Dr Simpson was awarded a gold medal for his "exhibit of the gross and microscopic changes in tularemia and for excellence of presentation." During the past two years he has contributed nine published papers dealing with the clinicopathologic studies of sixty four cases of tularemia in and about Dayton Ohio, and has been engaged with an investigation of the incidence of undulant fever in the same locality. Dr Simpson's book on Tularemia is just appearing from the press of Paul B Hoeber, Inc, New York City. His findings in sixty three cases of undulant fever were also just recently reported before the American Medical Association

The American Society of Clinical Pathologists takes great pride in numbering among its Fellows men of the caliber of Dr Walter Malcolm Simpson

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- 1 Billings J S Our Medical Literature Trans VII Intern Med Congress Lond 1 54-70 1881
- 2 Mayer Emil Medical Literature and Its Preparation Med Record N Y 87 1019 1021, July 1915
- Ailbutt, T C Notes on the Composition of Scientific Papers London Macmillan 1904
- McCrae Thomas The Use of Words J A M A 65 135 139 1915
- 3 Suggestions to Medical Authors Issued by the A M A Press Chic A M A [1914 (?) ]
- 4 Place F Bibliographic Style in Medical Literature Med Record N Y 83 157-160 1913

